MYELIN WATER AND PERFUSION IMAGING IN NORMAL APPEARING WHITE MATTER IN PATIENTS WITH MULTIPLE SCLEROSIS

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DECLARATION BY THE CANDIDATE

I hereby declare that the thesis entitled "**MYELIN WATER AND PERFUSION IMAGING IN NORMAL APPEARING WHITE MATTER IN PATIENTS WITH MULTIPLE SCLEROSIS**" submitted towards partial fulfillment of the requirements for the award of **MD in Radiodiagnosis** degree to BFUHS is a compilation of original work carried out by me under the supervision of **Dr. Kavita Saggar**, Professor & Head, Department of Radiodiagnosis, **Dr. Gagandeep Singh,** Professor & Head, Department of Neurology and **Dr. Ritu Dhawan Galhotra**, Professor, Department Radiodiagnosis. No part of this thesis has formed the basis for the award of any degree previously.

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Dedicated

to

my Family,

friends

&

Teachers

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ABBREVIATIONS

CIS	Clinically isolated syndrome
CBF	Cerebral blood flow
CBV	Cerebral blood volume
CSF	Cerebrospinal fluid
DIS	Disemmination in space
DIT	Disemmination in time
DSC	Dynamic susceptibility contrast
EDSS	Expanded disability status scale
MRI	Magnetic resonance imaging
MS	Multiple scleroosis
MTR	Magnetization transfer ratio
MVF	Myelin volume fraction
MWF	Myelin water fraction
MWI	Myelin water index
NAWM	Normal appearing white matter
PP-MS	Primary progressive multiple sclerosis
PR-MS	Progressive relapsing multiple sclerosis
QRAPMASTER	Quantification of relaxation times and proton density by multi-echo acquisition of a saturation-recovery using turbo spin-echo readout
RR-MS	Relapsing remitting multiple sclerosis
RIS	Radiologically isolated syndrome
SyMR	Synthetic Magnetic resonance imaging
SP-MS	Secondary progressive multiple sclerosis

ABSTRACT

INTRODUCTION: Multiple sclerosis is a chronic demyelinating disease of central nervous system (CNS) in which white matter (WM) lesions are visualized on T2 weighted MRI in most patients. Normal appearing white matter (NAWM) are areas of brain and spinal cord which appear 'normal' on standard clinical imaging however show demyelination on histologic studies. In this study we would evaluate Myelin volume and perfusion parameters to evaluate early demyelination in NAWM of patients with multiple sclerosis.

OBJECTIVE: Evaluation of Myelin volume and perfusion parameters in NAWM in patients of multiple sclerosis and its comparison with controls

METHOD OF COLLECTION OF DATA: It is a prospective study done on patients with multiple sclerosis based on the revised McDonalds criteria 2017,referred to MRI department of Radiodiagnosis Dayanand medical college, Ludhiana and compared with controls over a period of 1 and half years using SyMR software version 11.2.16 QRAPMASTER pulse sequence and DSC Perfusion imaging.

RESULTS: The mean Myelin volume (Myelin correlated component, Myc) in NAWM in MS patients was 31.95±1.67% as compared to controls which was 36.66±1.47% and the values were significantly lower in all regions of interest($p< 0.05$). The mean MyC is significantly correlated with disease duration, showing a negative correlation (Pearson correlation coefficient =0.48, r^2 =0.23). The mean normalized CBV of NAWM in MS patients is 0.40±0.07 as compared to controls which is 0.59±0.07. The mean normalized CBF of NAWM was 0.40±0.07 as compared to controls which was 0.55±0.07.The value of perfusion parameters is significantly lower in all regions of interest in cases as compared to controls.

CONCLUSION: Microstructural abnormalities occur in NAWM even before they are visible on routine MR sequences. Loss of myelin and reduced perfusion parameters precede plaque formation and structural atrophy in MS patients. Quantitatively assessing myelin loss could serve as a potential biomarker to determine the course of the disease. It could help clinicians initiate and titrate the treatment in MS patients early and to personalize treatment and monitor response in each patient

Keywords:, Multiple sclerosis, demyelination, NAWM, Myelin volume, perfusion, SyMRI, QRAPMASTER

TABLE OF CONTENTS

S. NO.	TOPIC	PAGES
I	INTRODUCTION	1-9
II	AIMS AND OBJECTIVES	10
III	REVIEW OF LITERATURE	11-17
IV	MATERIALS AND METHODS	18-26
V	OBSERVATIONS AND RESULTS	27-68
VI	DISCUSSION	69-75
VII	SUMMARY AND CONCLUSIONS	76-77
VIII	BIBLIOGRAPHY	78-84
	APPENDICES – I	
	- PROFORMA	
	- MASTER CHART	
	APPENDICES – II	
	- APPROVAL LETTER OF THESIS PLAN	
	- THESIS PLAN	

LIST OF TABLES

Table No.	Title	Page No.
1.	MRI Acquisition parameters	20
2.	Age and gender distribution of cases	27
3.	Age and gender distribution in controls	28
4.	Disease duration of patients, from the first onset of symptoms	30
5.	EDSS in patients with MS	31
6.	Average number of plaques in patients	32
7.	Myelin Correlated Component (MyC) in NAWM in regions of interest in patients with MS and controls	33
8.	Normalized CBV in all regions of interest and Mean values in cases and controls.	38
9.	nCBF in all regions of interest in NAWM in MS patients and controls.	40

LIST OF FIGURES

Fig. No.	Title	Page No.
Figure 1a	ROI placement at genu and splenium of corpus callosum	22
Figure 1b	ROI placement at b/l frontal and parietal region in NAWM	22
Figure 1c	ROI placement at b/l temporo-occipital region in NAWM	23
Figure 1d	ROI placement at MS plaque	23
Figure 2a	ROI placed at nawm in b/l frontal, parietal region in t1mprage post contrast sequence with orresponding ROI at dsc perfusion sequences similarly, ROI's were placed at b/l temporo occipital region, genu, body and splenium of corpus callosum	24
Figure 2b	Normalisation of values done by taking the ratio of perfusion parameters in NAWM to perfusion parameters of grey matter. the normalised value in this case is 34/79 i.e. 0.43	25
Figure 3	Age and gender distribution of cases	27
Figure 4	Gender distribution of patients and controls	28
Figure 5	Number of patients belonging to particular stage of MS	29
Figure 6	Average disease duration in patients	30
Figure 7	Demonstrating percentage of patients with EDSS less/more than 2	31
Figure 8	Average number of lesions in patients	32
Figure 9	Regional MyC in regions of interest	34
Figure 10	Mean MyC of all regions of interest	34
Figure 11	scatterplot of Mean MyC with disease duration	35
Figure 12	Scatterplot showing correlation of Mean MyC with no. of lesions	35
Figure 13	Scatterplot showing correlation of Mean MyC with EDSS	36
Figure 14	comparison of Myelin volume in Plaques, Normal Appearing white matter (NAWM) in patients and Normal white matter(NWM) in controls	37
Figure 15	showing comparison of Normalised CBV(nCBV) in all regions of interest in cases and controls	39

Figure 16	showing comparison for mean normalized CBV of cases and controls	39
Figure 17	showing comparison of Normalised CBF in all regions of interest in cases and controls	41
Figure 18	showing comparison of Mean normalized CBF of NAWM in cases and controls	41
Figure 19	showing scatterplot chart of correlation between normalized CBV and normalized CBF	42
Figure 20a	Control 1 with ROI in right frontal region(MyC= 39.7±1.9%)	43
Figure 20b	Control 1 with ROI in left frontal region (MyC=36.7±2.2%)	43
Figure 20c	Control 1 with ROI in left parietal region (MyC=35.2±2%)	44
Figure 20d	Control 1 with ROI in left parietal region (MyC=38±2.2%)	44
Figure 20e	Control 1 with ROI in right temporo-occipital region (MyC=41.2±3%)	45
Figure 20f	Control 1 with ROI in left temporo-occipital region (MyC=40.5±3.9%)	45
Figure 20g	Control 1 with ROI in splenium (MyC=43.2±5.9%)	46
Figure 20h	Control 1 with ROI in genu (MyC=40.2±3.5%)	46
Figure 21a	Case 1 with ROI in NAWM in right frontal region (MyC=24.4±3.2%)	47
Figure 21b	Case 1 with ROI in nawm in left frontal region (MyC=27.6±3.5%)	47
Figure 21c	Case 1 with ROI in NAWM in right parietal region (MyC=27.8±4.0%)	48
Figure 21d	Case 1 with ROI in nawm in left parietal region (MyC=25±3.1%)	48
Figure 21e	Case 1 with ROI in nawm in right temporo-occipital region (MyC=31.4±3.5%)	49

Figure 21f	Case 1 with ROI in NAWM in left temporo-occipital region (MyC=28±3.7%)	49
Figure 21g	Case 1 with ROI in genu of corpus callosum(MyC=35.6±6.5%)	50
Figure 21h	Case 1 with ROI in splenium of corpus callosum (MyC=38±5.0%)	50
Figure 22a	Case 2 with ROI in NAWM in right frontal region (MyC=25.7±3.2%)	51
Figure 22b	Case 2 with ROI in NAWM in left frontal region (MyC=29.9±2.8%)	51
Figure 22c	Case 2 with ROI in NAWM in right parietal region (MyC=27.2±3.1%)	52
Figure 22d	Case 2 with ROI in NAWM in left parietal region (MyC=28.9±2.4%)	52
Figure 22e	Case 2 with ROI in NAWM in right temporo-occipital region (MyC=31.2±2.5%)	53
Figure 22f	Case 2 with ROI in NAWM in left temporo-occipital region (MyC=31.8±3.3%)	53
Figure 22g	Case 2 with ROI in splenium (MyC=35.7±3.6%)	54
Figure 22h	Case 2 with ROI in genu (MyC=37.3±7.1%)	54
Figure 23a	ROI Placement in MS plaque in periventricular white matter in left parietal region	55
Figure 23b	ROI Placement in MS plaque in periventricular white matter in left frontal region	55
Figure 24a	Control 1 with ROI at grey matter right used for normalisation in right frontal region= 153	56
Figure 24b	Control 1 with normalised CBF right and left frontal region is 0.45 AND 0.47 respectively	57
Figure 24c	Control 1 with normalised cbf right and left parietal region is 0.41 and 0.40 respectively	58
Figure 24d	Control 1 with normalised CBF of genu and splenium is 0.40 and 0.41 respectively	59

Figure 24e	Control 1 with normalised CBF right and left temporo-occipital region is 0.43 and 0.40 respectively	60
Figure 25a	Case 1 with ROI at grey matter right frontal region used for normalisation= 108	61
Figure 25b	Case 1: Normalised CBV for right and left frontal region is 0.25 and 0.28 respectively normalised CBV for right and left parietal region is 0.25 and 0.25 respectively	62
Figure 25c	Case 1 with normalised CBV for right and left temporo-occipital regions is 0.25 and 0.31 respectively	63
Figure 25d	Case 1 with normalised CBV of genu and splenium and respectively 0.35 and 0.30 respectively	64
Figure 26a	Case 2 with ROI at of grey matter right frontal region used for normalisation = 66	65
Figure 26b	Case 2 with normalised CBF for right and left frontal region is 0.37 and 0.45 respectively normalised CBF for right and left parietal region is 0.39 and 0.34 respectively	66
Figure 26c	Case 2 with normalised CBF of genu and splenium and respectively 0.5 and 0.50 respectively	67
Figure 26d	Case 2 with normalised CBF for right and left temporo-occipital regions is 0.45 and 0.53 respectively	68

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory autoimmune demyelinating disorder of the central nervous system affecting approximately 2.8 million people worldwide [1,2]. It affects women approximately twice more as men [2]. It leads to severe cognitive and physical incapacitation primarily involving young adults. Initially characterized by reversible neurological deficits, eventually, it runs a progressive course [3]

Many potential triggers have been associated with its pathogenesis including EBV, Vitamin D deficiency, smoking, tobacco, and dietary habits. Genetic susceptibility also contributes to the pathogenesis of MS with a strong association of HLA DR2 and DR4 haplotypes. Activated T cells and macrophages enter the central nervous system through the disrupted blood-brain barrier (BBB) leading to the production of inflammatory cytokines which along with oxidative stress cause disruption of oligodendrocytes and subsequent demyelination. This leads to the formation of multiple grey and white matter plaques in the brain and spinal cord. [4]

The early disease course is marked by demyelination and varying degree of gliosis and axonal loss with perivenular lymphoid aggregates. The long-standing disease leads to cerebral atrophy and white matter loss. [5] Diffuse axonal injury of the normal appearing white matter also occurs outside the plaque. Hence NAWM refers to the normal looking yet diseased part of the brain which shows microstructural as well as perfusion abnormalities. [6]

4 patterns of multiple sclerosis have been defined:

1) Relapsing-remitting multiple sclerosis (RR-MS)
2) Secondary-Progressive multiple sclerosis (SP-MS)
3) Primary progressive MS (PP-MS)
4) Progressive- relapsing MS (PR-MS)

In 85% of cases, the course is RR-MS from the onset, and 15 % of cases present with a progressive course from onset-PPMS. RRMS over time eventually develops a secondary progressive disease pattern. [7]

Clinically isolated syndrome (CIS) is a monophasic clinical episode. It can either be a focal or a multifocal demyelinating event in CNS. It develops acutely for at least 24 hours and may or may not resolve. This episode should not be associated with fever or infection. For a person who is later diagnosed positive for MS using revised McDonald's criteria, this episode is considered as the first attack [8]

The first symptom ranges from optic neuritis, myelitis to brainstem syndrome, and focal supratentorial syndromes. Optic neuritis is the first manifestation in up to 20% of cases. It occurs in up to 50% of patients during their disease course. It usually presents with gradual onset vision loss, painful eye movements, and altered color vision. Blurred vision, flashing/flickering lights, blind spots, and other visual disturbances can be precipitated by the patient becoming overheated, which is known as Uhthoff's phenomenon. The clinical signs include reduced visual acuity, afferent papillary defect, scotoma, reduced contrast sensitivity.[9]

Myelitis presents with gradual onset of numbness, paresthesia, and weakness which may eventually progress to quadriparesis. Another phenomenon that has been described in MS is the Lhermittes phenomenon which is a shock-like sensation that typically occurs due to neck flexion and has been ascribed to lesions in the cervical cord. Brainstem syndromes can present with diplopia, oscillopsia, facial sensory loss, vertigo, and dysarthria. Typical findings include an isolated sixth nerve palsy, gaze-evoked nystagmus, or an internuclear ophthalmoplegia. Bilateral internuclear ophthalmoplegia is pathognomonic of MS. Uncommon first manifestations include headache, encephalopathy, isolated fatigue, or meningismus.[10]

Gradually as the disease progresses, one may develop changes in mentation, depression, loss of cognition, bladder and stool incontinence.

The average life span of patients with MS is 5-10 years lesser than the average [10].

To quantify degree of impairment in MS, John Kurtzke developed a disability score called the Expanded disability status scale (EDSS) in advance of his previous scale. It is based on the clinical examination done by a neurologist and describes disease progression in patients with MS, also it can be used to monitor the effectiveness of therapeutic interventions in clinical trials. It consists of an ordinal rating system ranging from 0 (normal neurological status) to 10 (death due to MS) in 0.5 increments interval (when reaching EDSS 1). The lower scale values of the EDSS measure impairments based on the neurological examination, while the upper range of the scale (> EDSS 6) measures the handicaps of patients with MS. The determination of EDSS 4 – 6 is heavily dependent on aspects of walking ability.[11]

There is no single lab parameter that can diagnose MS. Clinical history, signs and symptoms, CSF examination, and Magnetic Resonance Imaging (MRI) collectively have been used to diagnose MS.

The revised McDonald criteria is a revision to earlier criteria set up in 2010. 2017 McDonald criteria was intended to facilitate earlier and specific diagnosis simplify. It is used to clarify components of the 2010 McDonald criteria when multiple sclerosis is likely but not diagnosable. Conditions like Neuromyelitis Optica can be mistaken for Multiple sclerosis and therefore serological testing for Aquaporin 4 and Myelin oligodendrocyte Glycoprotein Antibody should be done in all patients with clinical features suggesting Neuromyelitis optica spectrum disorder.[12]

Brain Imaging with MRI should be obtained in all patients in whom the diagnosis of multiple sclerosis is being thought of.[13]

On MRI, a lesion is defined as hyperintensity on T2/proton density-weighted MRI which is at least 3 mm in long axis[14]

MRI sites typical for MS lesions are in

1) Periventricular white matter-T2/proton density hyperintense lesion abutting the lateral ventricles without white matter between or corpus callosum
2) Infratentorial- In the brainstem, cerebellar peduncle, and cerebellum
3) Juxtacortical- abutting the cortex without any white matter in between.
4) Spinal cord - hyperintense lesion on T2/STIR sequence or in 2 planes in T2 sequences[14]

The revised McDonald criteria incorporated cortical lesions in addition to juxtacortical lesions, in trying to fulfill the dissemination in space criteria.

In addition to this, the distinction between symptomatic and asymptomatic lesions was also removed in the revised criteria.[15]

The guiding principle of the diagnosis is that of dissemination in time (DIT) and dissemination in space (DIS). Dissemination in space has been defined as 1 or more lesions in 2 or more out of the 4 areas which are typical for MS. Dissemination in time is referred to as the development of new lesions with time or the simultaneous presence of enhancing and non-enhancing lesions. The T1 hypointense lesions which are chronic also called black holes do not help in fulfilling the criteria of dissemination in time.[10]

With a typical clinically isolated syndrome, fulfillment of clinical or MRI criteria for DIS, and no better explanation for the clinical presentation, demonstration of CSF oligoclonal bands in the absence of atypical CSF findings allow a diagnosis of multiple sclerosis to be made, even if the MRI findings on the baseline scan do not meet the criteria for DIT and in the absence of either a second attack or MRI evidence of a new or active lesion on serial imaging. This concludes that CSF oligoclonal band positivity can be substituted for dissemination in time. [15]

Radiological isolated syndrome(RIS) refers to imaging findings that strongly suggest a diagnosis of Multiple sclerosis, however, no definite neurological symptoms are present. It is postulated that 1/3rd of people

being diagnosed with RIS eventually were diagnosed with MS within 5 years of the diagnosis.[16]

The revised McDonald's criteria did not change much for the primary progressive course of Multiple sclerosis. It required:

At least 1 year of disability progression (retrospectively or prospectively determined) and did not depend on clinical relapse.[15]

Plus two of the following criteria:

• One or more T2-hyperintense lesions characteristic of multiple sclerosis in one or more of 4 characteristic sites of MS.

• Two or more T2-hyperintense lesions in the spinal cord

• Presence of CSF-specific oligoclonal bands[17]

Focal MS lesions are readily apparent on T2/FLAIR images. However, studies have shown that there is only a modest correlation of the number of lesions with a neurological deficit due to undetected changes in the normal appearing white matter of multiple sclerosis.[18]. The standardized MRI imaging protocol for MS includes: 3 plane scout, Sagittal FLAIR, Axial PD FSE/T2, Axial FLAIR, Axial T1 Pre-gadolinium, and Axial Post gad T1 weighted images. Saggital FLAIR images are more sensitive to early MS lesions in the corpus callosum, while axial FLAIR images are more sensitive to juxtacortical lesions. PD images are sensitive to infratentorial lesions, which may be missed on FLAIR images[19]. While it may seem obvious that patients who develop new lesions are worse off than those without new lesions, the association between clinical findings and radiological extent of involvement generally is poor. Much progress has been made in magnetic resonance so that the clinico-radiological dissociation has indeed proved to be a paradox. Thus, the relevance of normal-appearing brain tissue damage, residual brain volume, spinal cord damage, and cerebral plasticity had to be reiterated. [20]

The limitation of conventional magnetic resonance images is that scans are usually interpreted using the image signal contrast between tissues and not based on their absolute signal intensities.[21]

Various magnetic resonance (MR) techniques have been used to study the pathological evolution of demyelinating diseases, such as multiple sclerosis (MS). These are more sensitive and specific in evaluating non-lesional white matter abnormalities in the brain. Magnetization Transfer ratio(MTR) and diffusion tensor imaging(DTI) are the non-conventional MRI techniques that have been used to assess the NAWM in patients with MS.[22] MTR corresponds to the extent of demyelination with reduced MTR in NAWM which may correlate with the disease burden.[23] There is currently no recognized gold standard for myelin estimation, although myelin water fraction is one of the best validated and most commonly used quantitative measures for noninvasive assessment of myelin content in the brain [24]. Myelin imaging can potentially play an important role in the clinic, for predicting and monitoring responses to treatment. Myelin status is associated with changes in functional mobility following therapeutic exercises in patients with MS. However, myelin assessment is a difficult challenge, as it cannot be seen directly on MRI, which has a millimeter-scale resolution[25].

To validate a new myelin estimation technique, histological studies are powerful and considered as the standard criterion, but its significance is somewhat limited as histological staining is evaluated based on optical density contrasts and therefore does not directly quantify myelin. [26] Quantification of tissue parameters, such as relaxation rates and proton density, would provide an absolute scale; however, these methods have mainly been performed in a research setting. The development of rapid quantification, with scan times in the order of 6 minutes for full head coverage, has provided the prerequisites for clinical use. QRAPMASTER (quantification of relaxation times and proton density by multi-echo acquisition of a saturation-recovery using turbo spin-echo readout) method

for tissue relaxometry gives us absolute quantification of longitudinal relaxation time (T1), transverse relaxation time (T2), or their inverse relaxation rates (R1 and R2), and proton density (PD) provides an absolute scale and hence enables a more objective evaluation of disease. The model hypothesizes 4 compartments in the brain:

1. Myelin partial volume (or MVF)/Myelin correlated component(MyC) as used in our study with SyMR version 11.2.16)
2. Cellular partial volume
3. Free water partial volume
4. Excess parenchymal water partial volume.

It assumes that the relaxation behavior of each compartment contributes to the effective relaxation behavior of an acquisition voxel as a whole. Of these myelin partial volume and free water partial volume are automatically calculated in latest version of Sy MRI and is collectively presented as MVF. MVF (Myelin volume fraction) measured by this model has been validated by myelin staining of brain sections derived from cadavers. Absolute quantification of tissue properties has been previously reported for characterization of lesions and monitoring of treatment. Quantitative MRI enables direct comparison between brains objectively because it gives absolute numbers for relaxation behavior and water content. [27]

Hence the changes in MS NAWM are clinically relevant as they present very early in the course of the disease at the time of the first clinical presentation, and they correlate with a disability, cognitive impairment, and the degree of brain atrophy. [28]

The amount of blood perfusion in the brain is depicted as Cerebral Blood Flow (CBF) and is expressed as ml of blood per 100 gm of brain per minute. The typical CBF value for the whole brain is 60 ml of blood per 100 gm of brain tissue per minute. Cerebral blood volume (CBV) is defined as the amount of blood in a given volume of tissue and is expressed in (ml/100ml of tissue) or (ml/100 g of tissue).[29]

Cerebral perfusion can assess the oxygenation and the metabolism of different parts of the brain and could, indirectly, analyze the neurodegeneration occurring in this disease. Cerebral perfusion is a fundamental biological function which, at least under normal conditions, is coupled with parenchymal metabolism. In addition to cerebrovascular diseases, altered perfusion occurs in a variety of other diseases of the central nervous system. For example, in Alzheimer's disease, frontotemporal dementia, vascular dementia, and dementia with Lewy bodies, decreases in regional cerebral perfusion and blood volume have been reported and are consistent with decreased metabolic activity secondary to neurodegeneration.[30][31] The potential, therefore, exists for the measurement of perfusion to indicate the extent of neuronal and axonal loss or dysfunction in patients with MS.

Earlier studies measured relative perfusion values of lesions to the normal-appearing white matter(NAWM), however, perfusion NAWM, in reality, was abnormal making it an inadequate reference point.[32] Lymphocytic infiltration of vein walls without adjacent parenchymal inflammation, suggests that MS represents a form of vasculitis that could precede lesion development. It has also been proposed that endothelial activation of cerebral vessels leads to vascular occlusion that precedes cerebral parenchymal inflammation and demyelination; this would indicate that MS represents an ischemic pathophysiologic process. [33]

Although changes in the microvasculature of NAWM cannot be demonstrated anatomically with conventional MR imaging, assessment of regional cerebral hemodynamics is possible by using dynamic susceptibility contrast material–enhanced T2*- weighted perfusion MR imaging. Perfusion MR imaging methods involve the utilization of signal changes that accompany the passage of a tracer through the cerebrovascular system and enable the quantitative estimation of cerebral blood flow and the mapping of regional variations in cerebral microvasculature.[34]. Hence our study aims to further investigate if

microvascular abnormalities, which are related to the vascular inflammatory and occlusive changes, can be detected by measuring perfusion parameters using DSC-MRI in MS and their possible correlations with clinical parameters and neurological deficits.

AIMS AND OBJECTIVES

To evaluate Normal appearing white matter in patients of multiple sclerosis using Myelin and Perfusion imaging and to further compare the myelin volume and perfusion parameters with that of controls.

REVIEW OF LITERATURE

MYELIN IMAGING

C. Laule, I. M.Vavasour (2003) evaluated Thirty-three MS patients (24 relapsing-remitting, 8 secondary progressive, 1 primary progressive) and 18 controls. T2 relaxation data were obtained for each. 5 white matter and 6 grey matter structures were outlined in each of them. Myelin Water Fraction and water content were calculated for all regions of interest. The normal-appearing white matter (NAWM) water content was, on average, 2.2% greater than that from controls, with significant differences occurring in the posterior internal capsules, genu, and splenium of the corpus callosum, minor and major forceps**.** Myelin water fraction was 16% lower in NAWM than for controls, with significant differences in the major and minor forceps, internal capsules, and splenium ($p<0.05$). NAWM in MS has higher water content and lower myelin water fraction than normal white matter in healthy controls. The cause of the myelin water fraction decrease in NAWM was attributed to either diffuse edema, inflammation, demyelination, or any combination of these features[35]

Kitzler(2012) took a 26 patient cohort There were 16 patients with definite MS: 5 relapsing-remitting (RRMS), 6 secondary-progressive (SPMS), and 5 primary-progressive (PPMS). In addition, 10 clinically isolated syndrome (CIS) patients were recruited. The mean myelin water fraction was compared in different white matter regions between MS patients and healthy controls. Mean MWF in both total WM ($p< 0.01$) and NAWM ($p<0.01$) was significantly lower than the corresponding mean WM MWF for healthy controls. Across all MS courses, the mean MWF value was significantly lower in NAWM and lesions than in WM of controls.[36]

Shannon Kolind, Lucy Matthews(2012) evaluated the efficacy of whole-brain myelin water fraction (MWF) imaging in PPMS. It compared the MWF values in patients suffering from Primary Progressive Multiple sclerosis(PPMS) to healthy controls. It aimed to establish the relationship between MWF and clinical disability, regionally and globally throughout the

brain. It concluded that PPMS patients had significantly reduced global normal-appearing white matter MWF (6%, p=0.04) when compared to controls. Focal lesions showed lower MWF values than white matter in controls (p<0.001) and patients

(p<0.001). Along the white matter skeleton, MWF was diffusely reduced throughout the PPMS brain, with significant correlations between reduced MWF and increased clinical disability (more severe symptoms), as measured by the Expanded Disability Status Scale, within the corpus callosum and frontal, temporal, parietal and occipital white matter. [37]

Tobias Djamsched et al (2016) evaluated 17MS-patients and 14 age-matched healthy controls(HC)s using a 3T Magnetic Resonance Imaging (MRI) examination including MWI (8 slices, 12 minutes acquisition time), T2w and T1 pre and post gadolinium (GD) administration. Black holes , contrast-enhancing lesions , and T2 lesions were marked and registered to MWI. Additionally, regions of interest (ROI) were defined in the frontal, parietal, and occipital normal-appearing white matter (NAWM)/white matter (WM), the corticospinal tract, the splenium and genu of the corpus callosum in patients and HCs. Mean values of myelin water fraction (MWF) were determined for each ROI. Significant differences (p<0.05) of the MWF were found in all three different MS-lesion types, compared to the WM of HCs. The mean MWF values among the different lesion types were significantly differing from each other. Comparing MS patients vs. HCs, significant (p<0.05) difference of the MWF in all measured ROIs except genu and splenium of corpus callosum.No age, sex, disability score, and disease duration dependency were found for the NAWM MWF.[38]

A. Hagiwara, M. Hori, K. Yokoyama(2017) evaluated Twenty patients with relapsing-remitting MS with a combined total of 149 chronic plaques were analyzed. Myelin volume fraction was calculated based on simultaneous tissue relaxometry. Intracellular and CSF compartment volume fractions

were quantified via neurite orientation dispersion and density imaging. Axon volume fraction and g-ratio were calculated by combining these measurements. Myelin and axon volume fractions and g-ratio were measured in plaques, periplaque WM, and normal-appearing WM. All metrics differed significantly across the 3 groups ($P < .001$, except $P < .027$ for g-ratio between periplaque WM and normal-appearing WM). Those in plaques differed most from those in normal-appearing WM. The percentage changes in plaque and periplaque WM metrics relative to normal-appearing WM were significantly larger in absolute value for myelin volume fraction[39]

Irene M Vavasour, Sophie C Huijskens [2018] evaluated 11 subjects with RRMS and 4 controls which were scanned on a 3T MRI at baseline and long-term follow-up. For every voxel, MWF, ieGMT$_2$, and T$_1$ were obtained. In MS subjects, NAWM MWF means decreased by 8% ($p = 0.0016$). No longitudinal changes were measured in T$_1$ or ieGMT$_2$. There was no relationship between change in any MR metric and change in EDSS. The decreases we observed in MWF suggest that changes in myelin integrity and loss of myelin may be occurring diffusely and over long periods in the MS brain. The timescale of these changes indicates that chronic, progressive myelin damage is an evolving process occurring over many years.[40]

Joon Yul Choi [1], In Hye Jeong(2019) conducted a study on twenty-five MS patients and 18 healthy controls (HC). MWFs of ViSTa were significantly lower in the MS patients than those in the HC in all masks ($P < 0.001$). GRASE-MWI results revealed reduced MWFs only in global WM, genu, and optic radiation. ViSTa-MWI had higher statistical powers than that of GRASE-MWI (power: ViSTa = 99.2 ± 1.6% and GRASE = 75.5 ± 31.0%; In early-stage MS, MWFs of ViSTa were significantly lower than those of the HC in all masks except for centrum semiovale; however, MWFs of GRASE MWI were significantly lower only in optic radiation. Disease duration was correlated with both MWIs (ViSTa; r = -0.437 and GRASE; r

= -0.445). ViSTa and GRASE MWFs were significantly correlated in the HC (r = 0.664) and MS (r = 0.768).[41]

Laetitia Saccenti, Akifumi Hagiwara (2020)evaluated the correlation between synthetic MRI myelin volume fraction (SyMRIMVF) and myelin fraction estimated by other techniques, i.e., magnetization transfer saturation (MTsat), T1-weighted images divided by T2-weighted images (T1w/T2w), and radial diffusivity (RD) in patients with MS. SyMRIMVF was found useful in evaluating white matter in MS patients, with relevant results as compared to other well-studied techniques. Moreover, it presented better sensitivity for the detection of the difference between the plaque or periplaque white matter and NAWM. It showed good repeatability and reproducibility across different in-plane resolutions and scanners. It showed a correlation with disease duration however no significant correlation was found between EDSS and SyMRIMVF.[42]

PERFUSION STUDIES

Multiple sclerosis is an immune-mediated inflammatory and neurodegenerative process. There has been interest in vascular pathology contributing to MS based on the fact that lesions develop around central veins with lymphocytic infiltration of vein walls. Ischemia could occur in MS owing to the microvascular occlusive changes. Hence perfusion changes imply assessing regional hemodynamic variation using dynamic susceptibility contrast material T2* weighted perfusion imaging. Various studies have been done to evaluate perfusion changes in NAWM which may precede plaque formation in MS.

W Rashid et al(2003) evaluated 60 patients (21 relapsing-remitting, 14 secondary progressive, 12 primary progressive, 13 benign) and 34 healthy controls. Statistical parametric mapping (SPM '99) was used to investigate regional variations in perfusion in both GM and WM. Global WM perfusion was derived by segmenting WM from images using T1 relaxation times. Regions of lower perfusion in predominantly GM were observed in the primary and secondary progressive cohorts, particularly in the thalamus.

Increased WM perfusion was seen in relapsing-remitting and secondary progressive cohorts.[43]

Sumita Adhya, a Glyn Johnson et al(2006) aimed to determine the regional pattern of tissue perfusion in the normal-appearing white matter (NAWM) of patients with primary-progressive (PP), relapsing-remitting (RR) multiple sclerosis (MS), and healthy controls, and to investigate the association between perfusion abnormalities and clinical disability. Using dynamic susceptibility contrast (DSC) perfusion MRI at 3 T, 22 patients with clinically definite MS, 11 with PP-MS and 11 with RR-MS, and 11 age- and gender-matched healthy volunteers were included. Absolute cerebral blood flow (CBF), cerebral blood volume (CBV) and mean transit time (MTT) were measured in the periventricular, frontal, occipital NAWM, and in the splenium of the corpus callosum. Compared to controls, CBF and CBV were significantly lower in all NAWM regions in both PP-MS patients and RR-MS patients and lower CBV in the periventricular and frontal NAWM (p values: 0.0029 and 0.022). EDSS was significantly correlated with the periventricular CBF ($r<0.48$, $p= 0.0016$) and with the periventricular and frontal CBV ($r<0.42$, $p= 0.015$; $r<0.35$, $p= 0.038$, respectively). This study suggests that the hemodynamic abnormalities of NAWM have clinical relevance in patients with MS.[32]

Miho Ota Et al(2013)evaluated, who 27 patients with MS and 24 healthy volunteers who underwent magnetic resonance imaging and pCASL to assess CBF. Differences in CBF between the two groups and the relationships of CBF values with the T2-hyperintense volume were evaluated. Compared to the healthy volunteers, reduced CBF was found in the bilateral thalami and right frontal region of the MS patients. The volume of the T2-hyperintense lesion was negatively correlated with regional CBF in some areas, such as both thalami. Results suggest that demyelinated lesions in MS mainly have a remote effect on the thalamus and that the measurement of CBF using ASL could be an objective marker for monitoring disease activity in MS.[44]

Maxim Bester et al (2015) evaluated 33 patients with relapsing-remitting MS using 3T MRI scans including dynamic susceptibility contrast perfusion-weighted MRI. Cerebral blood flow (CBF) and cerebral blood volume (CBV) were measured in normal-appearing white matter. Patients were stratified in a high- and low-inflammatory group according to the number of new contrast-enhancing lesions.13 were classified as high-inflammatory. Compared to low-inflammatory patients, the high-inflammatory group demonstrated significantly higher CBV ($p = 0.001$) and CBF ($p = 0.014$) values.[45]

Meng Law et al(2016) Dynamic susceptibility contrast-enhanced T2*-weighted MR imaging was performed in 17 patients with RRMS) and 17 control patients. Absolute cerebral blood volume (CBV), absolute cerebral blood flow (CBF), and mean transit time (MTT) (referenced to an arterial input function by using an automated method) were determined in periventricular, intermediate, and subcortical regions of NAWM at the level of the lateral ventricles. Each region of NAWM in patients with RR-MS had significantly decreased CBF ($p< .005$) and prolonged MTT ($p<.001$) compared with the corresponding region in control patients[46].

Robert M. D'Ortenzio et al(2017)compared pseudo-continuous Arterial Spin Labeling CBF to the Bookend technique in a prospective cohort of 19 healthy controls, 19 relapsing-remitting MS subjects without cognitive impairment and 20 relapsing-remitting MS subjects with cognitive impairment on a voxel-wise and Brodmann regional basis. Voxel-wise paired T-tests revealed no significant CBF differences between techniques after normalization of global mean intensities. Highest Pearson correlations were observed in deep GM structures (average $r = 0.71$ basal ganglia, and $r = 0.65$ thalamus) but remained robust for cortical GM, WM and WML (average $r = 0.51, 0.53, 0.54$ respectively). [47]

Ping Yin et al (2018) evaluated 30 patients with multiple sclerosis (MS) who underwent conventional magnetic resonance imaging (MRI) and DCE-MRI with a 3.0 Tesla MR scanner. An extended Tofts linear model

was used to quantitatively measure MR imaging biomarkers. The histogram parameters and correlation among imaging biomarkers, EDSS scores, and disease duration were also analyzed. It was observed that the MR imaging biomarkers volume transfer constant (K^{trans}), the volume of the extravascular extracellular space per unit volume of tissue , fractional plasma volume , cerebral blood flow (CBF), and cerebral blood volume (CBV) of contrast-enhancing (CE) lesions were significantly higher ($P < 0.05$) than those of nonenhancing lesions and normal-appearing white matter (NAWM) regions.[48]

MATERIALS AND METHODS

Source of data

It was a prospective study of patients undergoing MRI Brain with contrast in Department of Radiodiagnosis Dayanand medical college and Hospital, Ludhiana for one and a half year Those with a proven diagnosis of Multiple sclerosis according to revised McDonalds criteria 2017 were taken as cases and those who did not have any white matter abnormality on imaging were taken as controls.

METHOD OF COLLECTION OF DATA

INCLUSION CRITERIA:

- All patients with multiple sclerosis based on revised McDonalds criteria 2017
- Controls with no white matter disease on MR imaging

EXCLUSION CRITERIA:

- Patients with alternative diagnosis.
- Any contraindications to MRI(patients with cardiac pacemakers, claustrophobic patients)
- Anyone unwilling to participate in the study.

Preparation of the patients

Consent was obtained from each patient for inclusion in the study. The relevant clinical history, imaging records and laboratory investigations were recorded on a proforma. Before starting the scan, the procedure was explained to the patient in his/her vernacular language to allay anxiety. Patients who fulfilled the revised McDonalds criteria 2017 were taken as cases and those patients who did not have any white matter abnormality were taken as healthy controls. Prior to imaging neurological examination was done and disability was calculated using EDSS score.

MRI PROTOCOL:

All patients underwent MR imaging using the following protocol:

Conventional MR imaging was done on multiplanar, multisequential scan using a SIEMENS MAGNETOM SKYRA 48 CHANNEL 3 Tesla MRI using 20 channel head coil.

These patients underwent following sequences

Axial T2 imaging(Propellar) and corresponding T1 imaging, T2 FLAIR, T2 FLAIR Saggital, Diffusion weighted imaging with a b value of 1000m2/sec was performed using appropriate parameters.

Dynamic susceptibility contrast MRI was performed with an intravenous dose of 0.1mmol/kg body weight of a GD-based contrast agent at a rate of 4 ml/sec for DSC followed by a flush of 10 ml saline injected at same rate

Finally a post contrast acquisition of the entire brain which included post contrast T1 images and post contrast axial MPRAGE was carried out.

Myelin imaging was done using QRAPMASTER pulse sequence using SyMR software version 11.2.16.

The sequences were obtained with following parameters:

Table 1 : MRI ACQUISITION PARAMETERS

Sequence	T1 Axial	T2 Axial	T2 FLAIR Axial	T2 FLAIR Saggital	DWI	T1 Post Contrast	SWI	QRAPMASTER	Perfusion Axial
Acquisition Time	1.22min	1.28min	2.08 min	3.42 min	2.03 min	5.38 min	1.28min	5 min 15 sec	1.42min
Orientation	Transverse	Transverse	Transverse	Saggital	Transverse	Transverse	Transverse	Transverse	Transverse
Resolution Matrix	204x256	269x512	227x320	227x320	160x160	256x256	163x224	208x320	128x128
No. of slices	27	27	27	27	27	176/slab	60/slab	30	20
FoV Read(mm)	220	220	220	220	220	230	220	230	220
FoV phase (%)	87.5	87.5	87.5	87.5	100	100	91.1	81.3	100
Slice thickness (mm)	4mm	4mm	4mm	4mm	4mm	1mm	2.5mm	4mm	4mm
Slice Gap(mm)	1.2mm	1.2mm	1.2mm	1.2mm	1.2mm	-	-	1mm	1.2mm
Repitition Time(msec)	2000msec	5060 msec	9000msec	9000msec	5500msec	2200msec	27msec	4350msce	1600msec
TE(msec)	8.6msec	91msec	81msec	81msec	64msec	2.48msec	20msec	TE1-23 msec TE2- 101 msec	30msec
Flip Angle	150	120	130	130	180	8	15	150	90
Flow Compression	No	Read	No	No	-	No	Yes	No	

IMAGE PROCESSING AND DATA ANALYSIS-

Data post processing was performed on workstation using SYNGOVIA MR perfusion software and Synthetic MR software version 11.2.16 for image analysis. Synthetic T1w and T2w images were created using post-processing TE of 23 msec and 101msec and TR of 4350 msec, on SyMRI software based on R1, R2, and PD maps.

ROI PLACEMENT

For QRAPMASTER sequence, ROIs(region of interest(0.04-0.10ml) were located in Normal appearing white matter(NAWM) . Atleast 3 ROIs were placed in right and left frontal, parietal and temporo occipital regions respectively as well as in genu and splenium of corpus callosum to obtain reproducibility. ROIs were so placed to avoid arterial or venous structures in NAWM, particularly in the periventricular and subcortical regions. Effort was made to exclude the ROI placement in or near the MS plaques. MyC(Myelin correlated component) was then calculated. MyC is the mean myelin volume in that ROI and is expressed in percentage. The obtained values were then averaged. (Various studies which used SyMRI sofrware have mentioned this as MVF)

ATleast 4 ROI's were also placed at the MS Plaques. The burnt out lesions or cystic lesions were avoided. MyC values were noted. The values were then subsequently averaged.

For DSC, 3 ROIs(4-7 mm2) were located in NAWM in right and left frontal, parietal and temporo occipital regions respectively as well as genu, body and splenium of corpus callosum each which were subsequently averaged. The values were normalized by placing 3 ROI at contralateral grey matter later averaged. ROIs were so placed to avoid arterial or venous structures in NAWM, particularly in the periventricular and subcortical regions. Quantitative analysis was performed with mean value of ROI of that region divided by the mean value of reference ROIs for grey matter. Hence normalized values of CBF and CBV were obtained.

1. MYELIN IMAGING

METHOD OF ROI PLACEMENT IN NAWM

Figure 1a: ROI placement at genu and splenium of corpus callosum

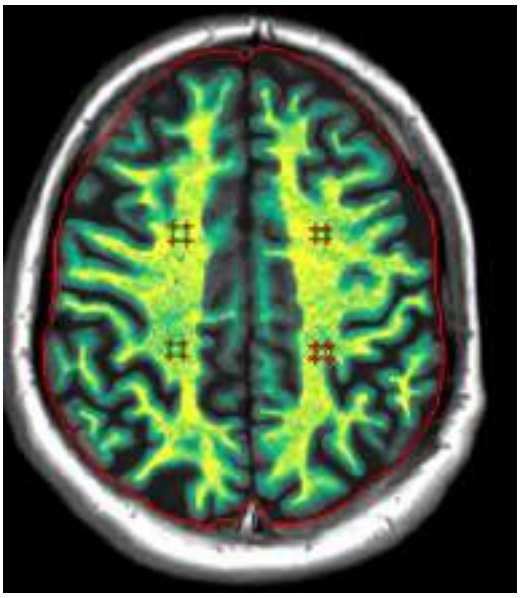

Figure 1b: ROI placement at b/l frontal and parietal region in NAWM

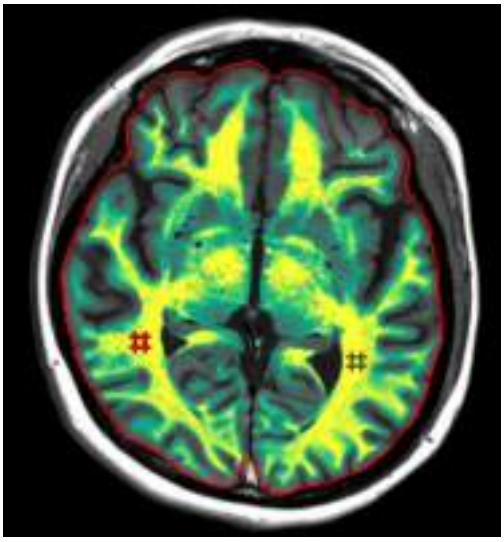

Figure 1c: ROI Placement at b/l temporo occipital region in NAWM

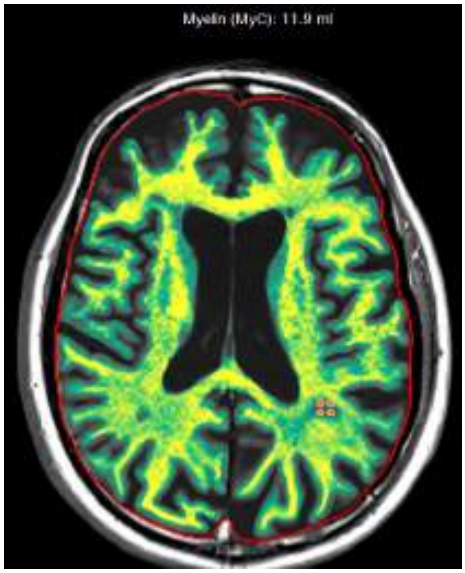

Figure 1d: ROI Placement at MS plaque

2. PERFUSION IMAGING

METHOD OF ROI PLACEMENT IN NAWM

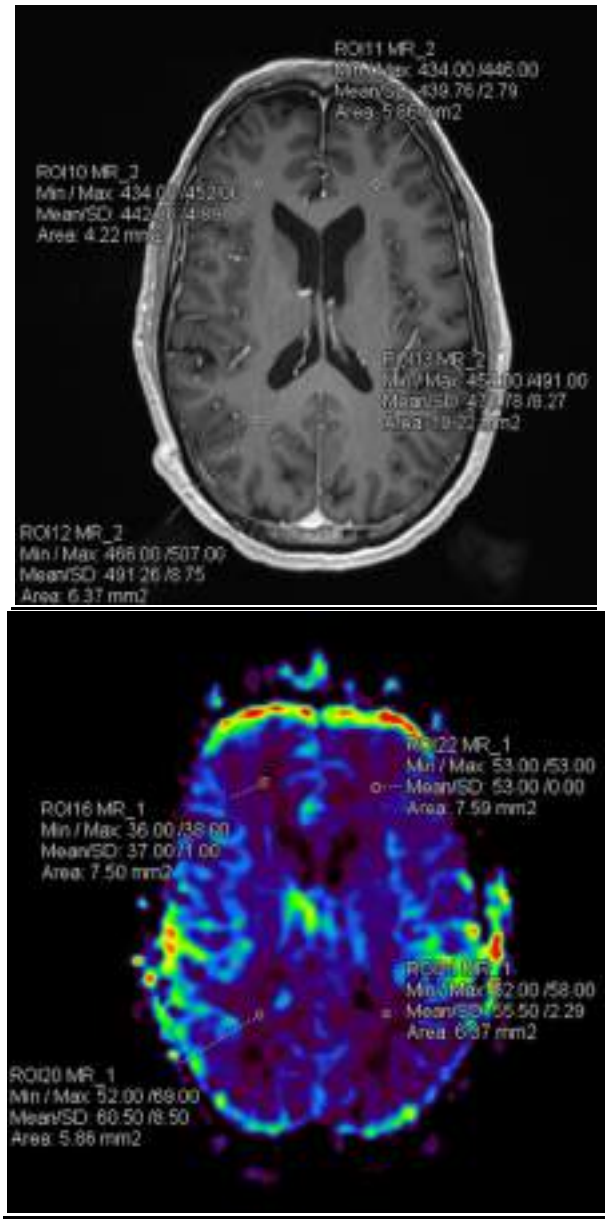

Figure2a: ROI Placed at NAWM in b/l frontal, parietal region in t1mprage post contrast sequence with corresponding ROI at DSC perfusion sequences

Similarly, ROI's were placed at b/l temporo occipital region, genu, body and splenium of corpus callosum

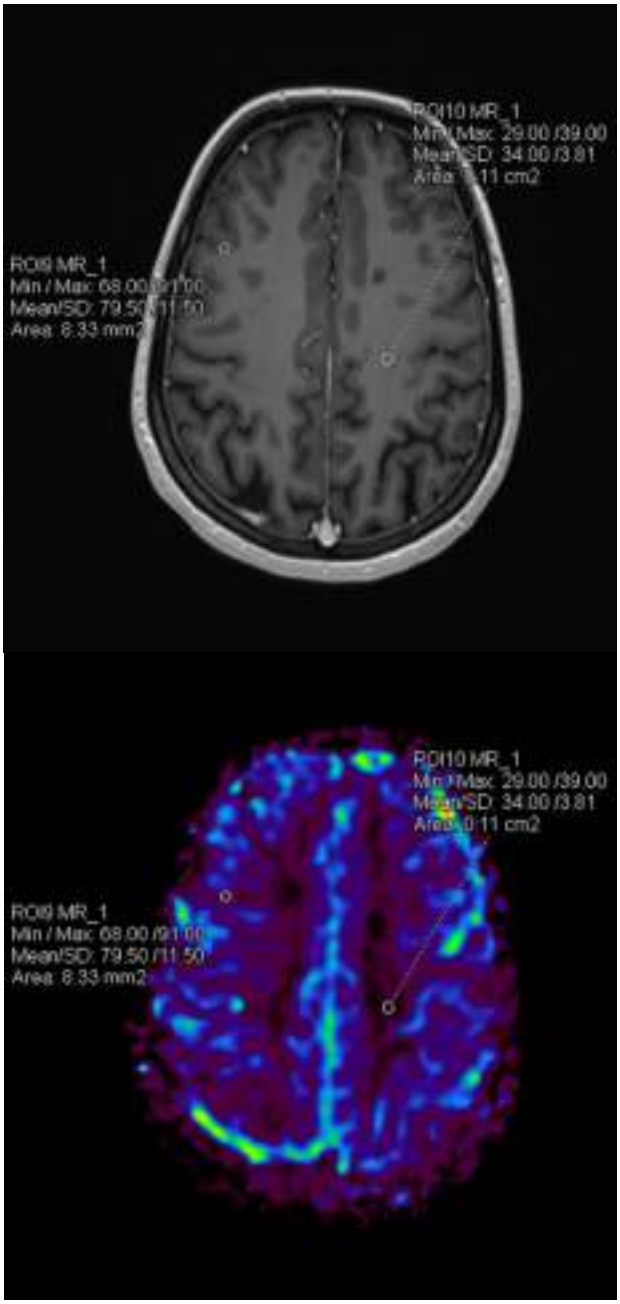

Figure 2b: Normalisation of values done by taking the ratio of perfusion parameters in NAWM to perfusion parameters of grey matter. The normalised value in this case is 34/79 i.e. 0.43

DURATION OF STUDY:

The study was conducted over a period of 1 and a half year.

OUTCOME:

- MRI was performed on proven MS patients according to revised McDonalds criteria 2017.
- Myelin Correlated component(MyC) was calculated in NAWM in MS Patients and controls using QRAPMASTER pulse sequence
- Normalised perfusion parameters (CBF and CBV)were calculated in NAWM of patients with multiple sclerosis and controls using DSC MRI.
- The above parameters were then compared to NAWM of controls to assess early demyelinating changes in the areas which appear otherwise normal on standard MR imaging.

STATISTICAL ANALYSIS:

Data will be described in terms of range; mean ±standard deviation (±SD), median, frequencies (number of cases) and relative frequencies (percentages) as appropriate. Comparison of quantitative variables between the study groups will be done using Student t-test and Mann Whitney U test for independent samples for parametric and non-parametric data respectively. For comparing categorical data, Chi square ($\chi 2$) test will be performed and exact test was used when the expected frequency is less than 5. A probability value (p value) less than 0.05 will be considered statistically significant. All statistical calculations will be done using SPSS (Statistical Package for the Social Science) SPSS 21 version statistical program for Microsoft Windows.

OBSERVATION AND RESULTS

1. Demographic data

20 patients were evaluated in this study between the period of September 2020-November 2021. The age range of patients was from 14-50 years, including 12 females and 8 males as shown in Table2, Figure 3. All patients were diagnosed cases of Multiple sclerosis according to the revised McDonalds criteria 2017.

18 healthy controls were included in the study ranging from 18-62 years, out of which 9 were males and 9 females as shown in Table 3, Figure 4 All patients tolerated MRI examination without any adverse reaction to contrast media

			SEX			
		FEMALE		MALE		TOTAL
AGE GROUP	0-10	0	0%	0	0%	0
	10-20	2	16.6%	0	0%	2
	20-30	2	16.6%	4	50%	6
	30-40	7	58.3%	3	37.5%	10
	40-50	1	8.3%	1	12.5%	2
TOTAL		12	100%	8	100%	20

Table 2 showing age and gender distribution of cases

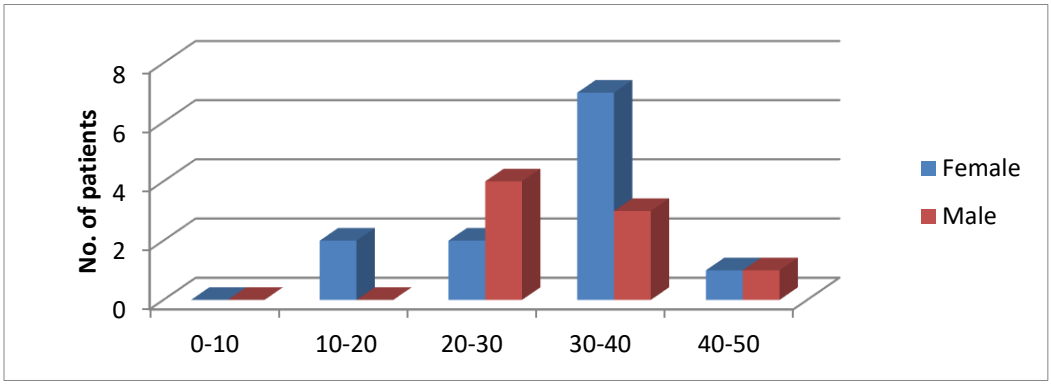

Figure 3 showing age and gender distribution of cases

			SEX			TOTAL
		FEMALE		MALE		
AGE GROUP	0-10	0	0%	0	0%	0
	10-20	2	22.2%	0	0%	2
	20-30	2	22.2%	3	33.3%	5
	30-40	1	11.1%	2	22.2%	3
	40-50	1	11.1%	1	11.1%	2
	50-60	1	11.1%	3	33.3%	4
	60-70	2	22.2%	0	0%	2
TOTAL		9	100%	9	100%	18

Table 3 showing age and gender distribution in controls

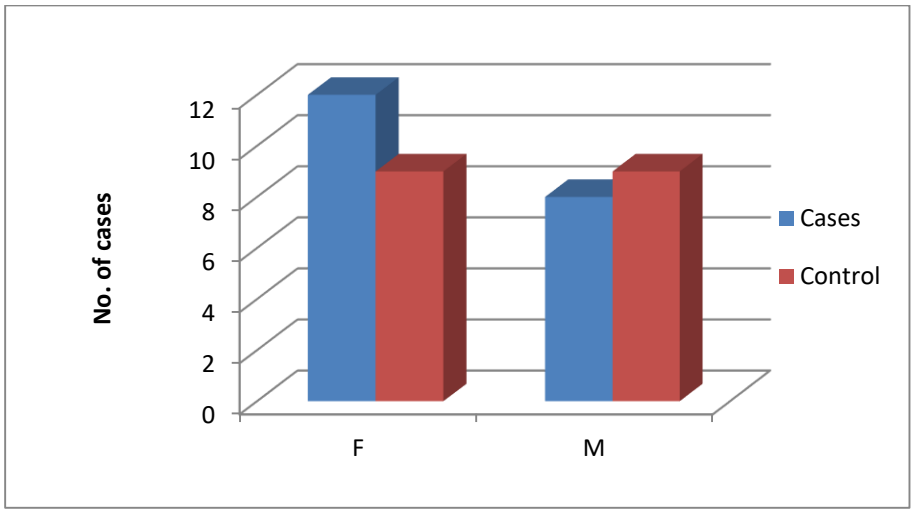

Figure 4 showing gender distribution of patients and controls

2. STAGE OF PROGRESSION

Out of the 20 patients, 19 patients belonged to Relapsing remitting type of MS (RRMS), and 1 patient belonged to Secondary progressive type of MS(SPMS). (Figure 5)

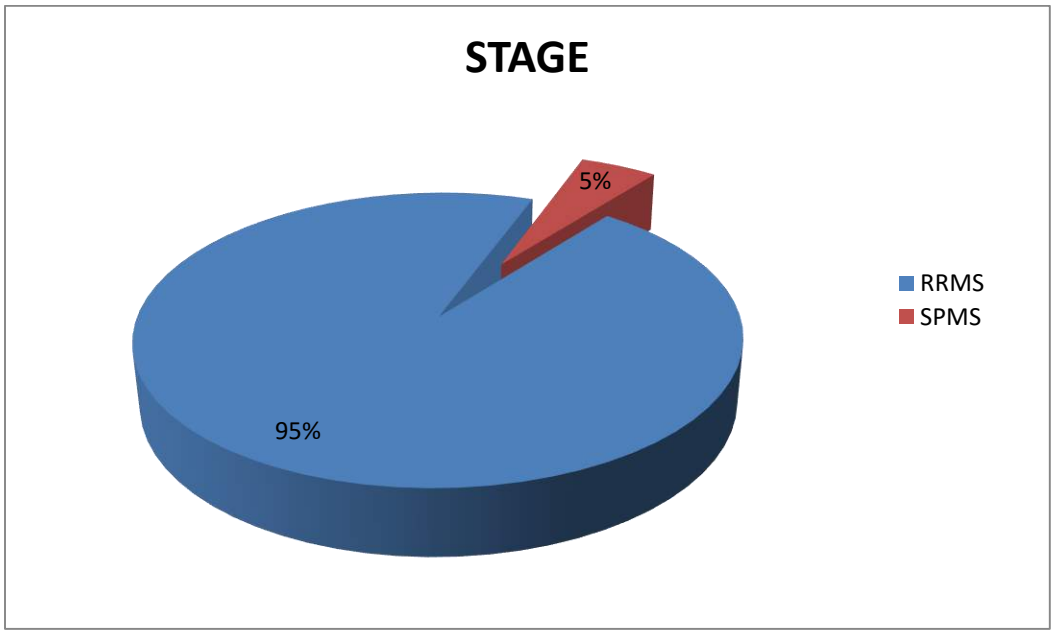

Figure 5 Pie chart showing number of patients belonging to particular stage of MS

3. DISEASE DURATION

The disease duration of patients ranged from 4 months to 10 years. The disease duration of patients is given in Table 4, Figure 6

Duration	No. of cases	Percentage
< 1YR	5	25.0%
1-5YR	7	35.0%
> 5YR	8	40.0%
Total	20	100.0%

Table 4 showing disease duration of patients, from the first onset of symptoms

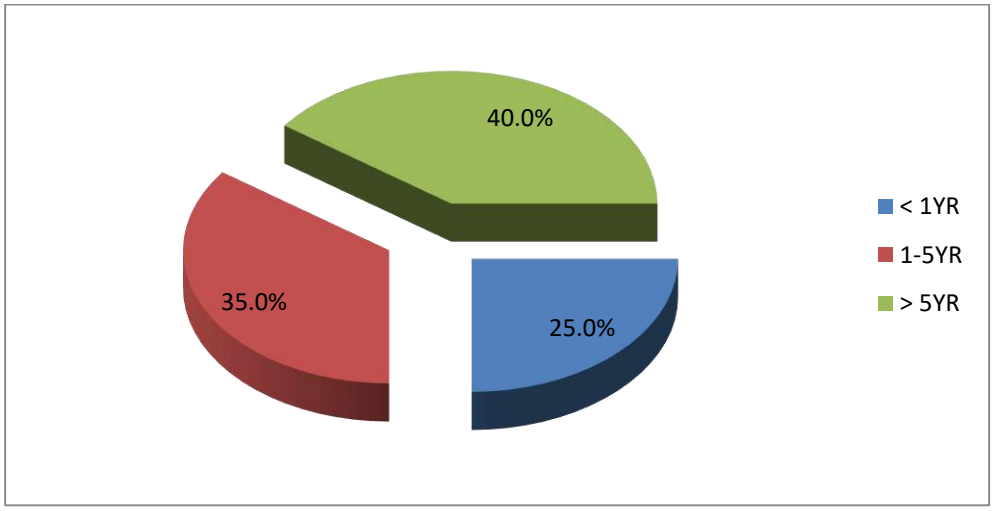

Figure 6 Pie chart showing average disease duration in patients

4.EDSS (Expanded disability status scale)

EDSS was calculated in each patient. EDSS ranged from 1-6.5, with a mean of 2.8. The EDSS distribution in patients is given in Table 5 and Figure 7

EDSS	No. of cases	Percentage
1	4	20.0%
2	8	40.0%
3	4	20.0%
5.5	1	5.0%
6	2	10.0%
6.5	1	5.0%
Total	20	100.0%

EDSS	No. of cases	Percentage
< 2	12	60.0%
> 2	8	40.0%
Total	20	100.0%

Table 5 showing EDSS in patients with MS

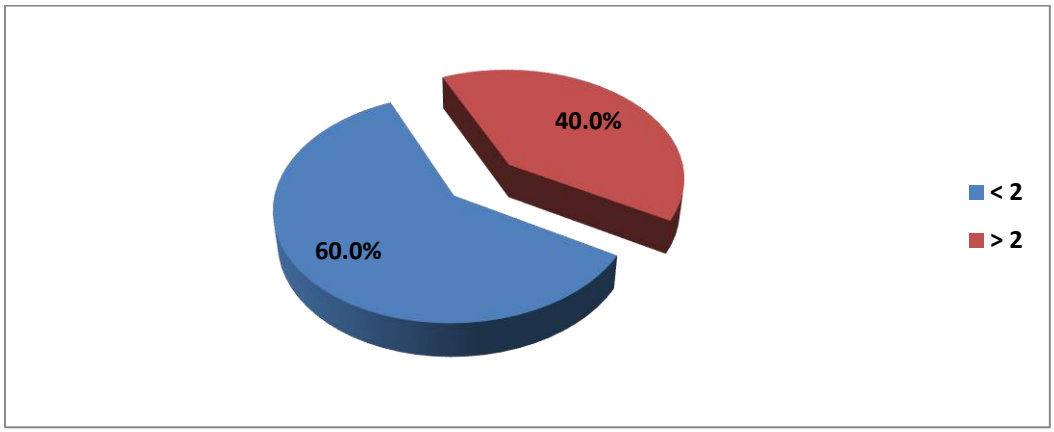

Figure 7 Pie chart demonstrating percentage of patients with EDSS less/more than 2

4. LESION LOAD

The number of lesions were calculated in each patient and further classified shown in Table 6, Figure 8

LESION	No. of cases	Percentage
< 20	9	45.0%
21-40	6	30.0%
41-60	2	10.0%
61-80	1	5.0%
> 80	2	10.0%

Table 6 showing average number of plaques in patients

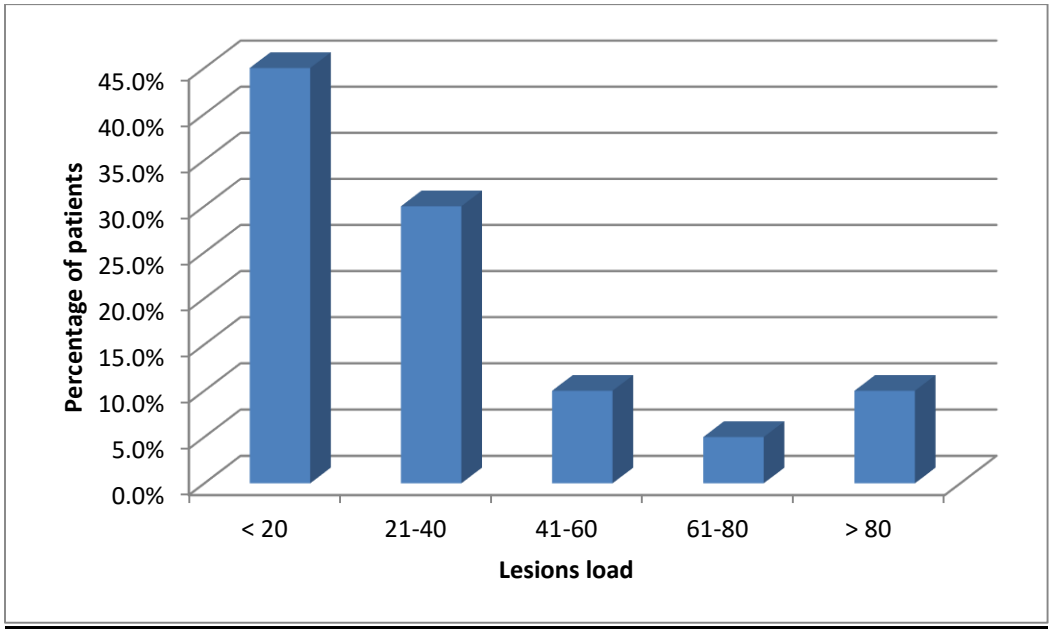

Figure 8 showing average number of lesions in patients

6.MYELIN IMAGING

6a. Quantitative Myelin volume (MyC) measurements

Quantitative Myelin correlated component (MyC)measurements in percentage were obtained in all 20 patients and 18 controls in Normal appearing white matter in 8 regions of Interest, which included Right and Left Frontal, Parietal, TEMPORO-OCCIPITAL White matter and genu and splenium of corpus callosum as shown in Table 7, Figure 9 and Figure 10. The mean MyC of patients with MS was 31.95±1.67% while that of controls was 36.6±1.47%. The mean MyC was 14.8% lower than that of controls.

	Cases		Control		t	p-value	Difference		
	MyC	SD	MyC	SD			Mean	SD	% age
R.FRONTAL	30.13	2.78	36.38	2.46	-7.304	0.000	-6.25	0.86	-20.8
L. FRONTAL	29.64	2.32	36.30	2.61	-8.323	0.000	-6.66	0.80	-22.5
R. PARIETAL	29.89	3.00	35.05	1.81	-6.329	0.000	-5.17	0.82	-17.3
L.PARIETAL	29.90	2.88	34.63	1.68	-6.106	0.000	-4.74	0.78	-15.8
R.TEMPORO-OCCIPITAL	32.26	2.26	36.47	2.27	-5.718	0.000	-4.21	0.74	-13.1
L.TEMPORO-OCCIPITAL	30.72	1.76	36.27	2.21	-8.591	0.000	-5.55	0.65	-18.1
GENU	38.15	2.49	40.22	2.28	-2.674	0.011	-2.08	0.78	-5.4
SPLENIUM	34.90	2.29	37.97	2.58	-3.894	0.000	-3.07	0.79	-8.8
MEAN MyC	**31.95**	**1.67**	**36.66**	**1.47**	**-9.212**	**0.000**	**-4.72**	**0.51**	**-14.8**

Table 7 showing Myelin Correlated Component (MyC) in NAWM in regions of interest in patients with MS and controls

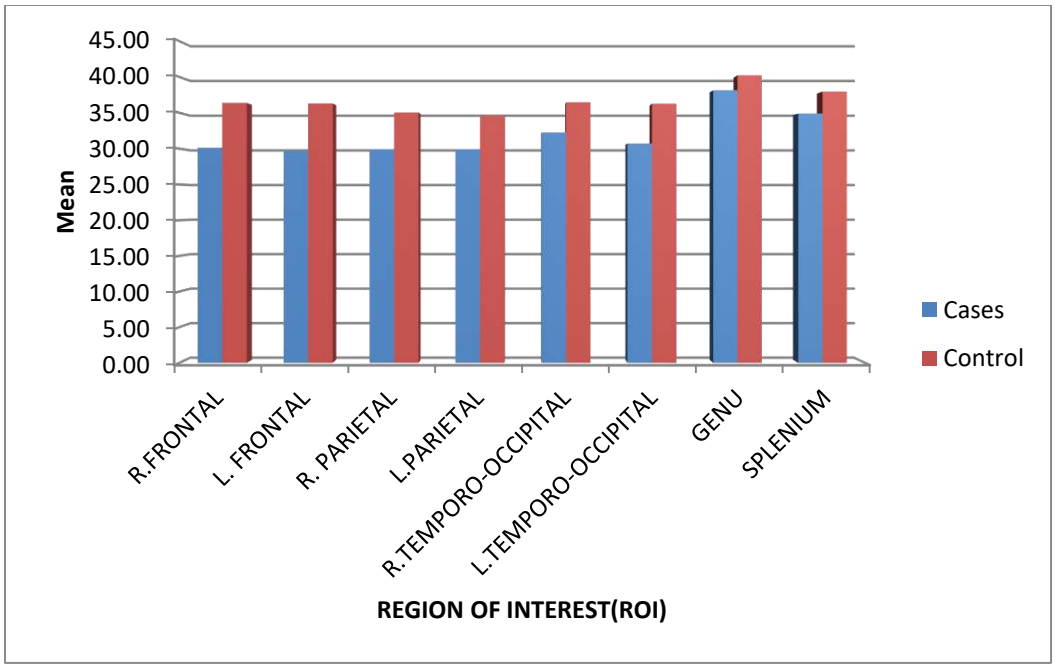

Figure 9 showing Regional MyC in regions of interest

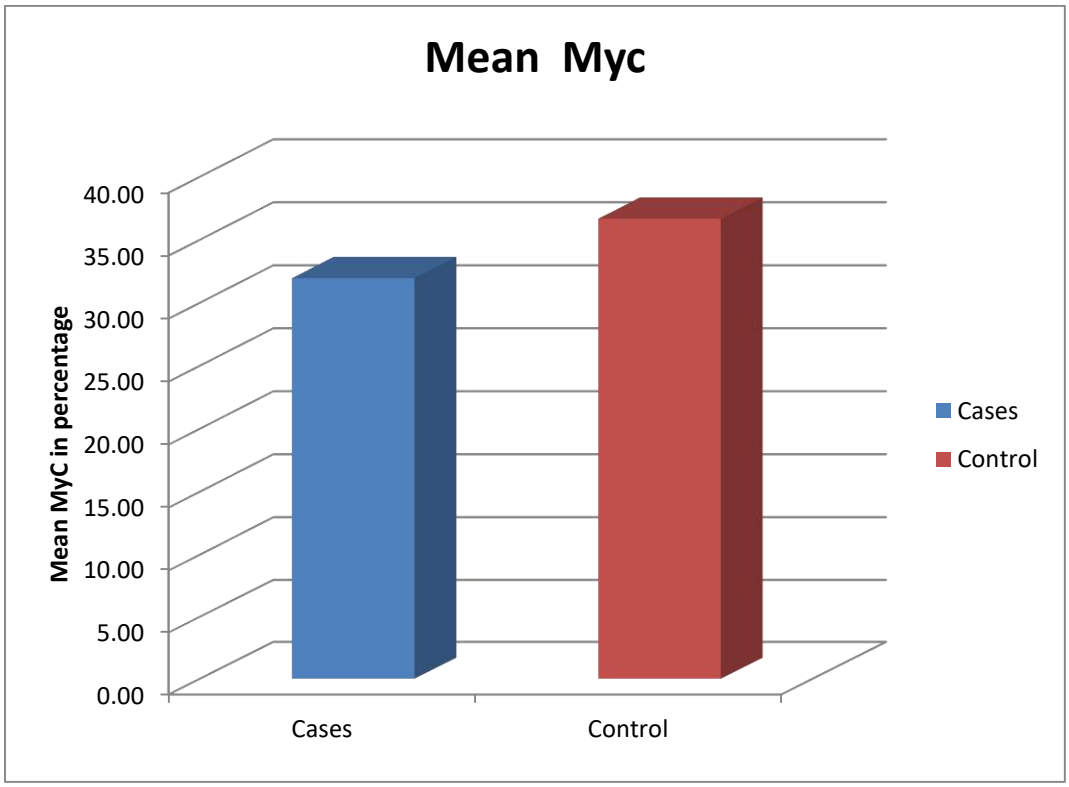

Figure 10 showing Mean MyC of all regions of interest

6b. Correlation between MyC and disease duration

The Pearson correlation coefficient of Mean MyC to disease duration was 0.48 with $r^2 = 0.23$, p= 0.03.(Figure 11)

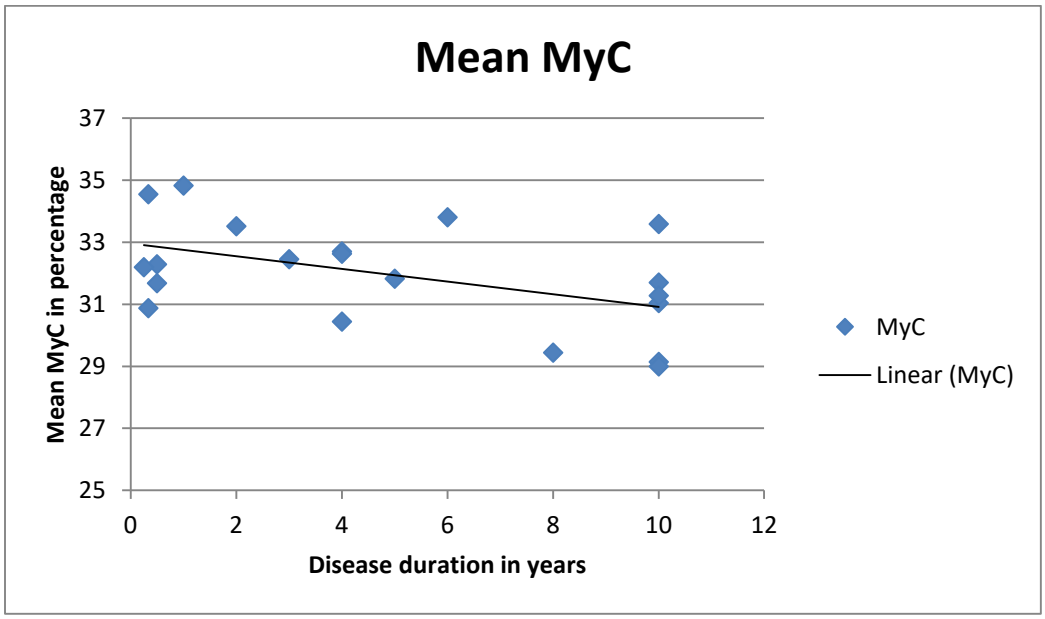

Figure 11 showing scatterplot of Mean MyC with disease duration

6c : Correlation between MyC and No. of lesions

The Pearson correlation coefficient of MyC with number of lesions was 0.68 with r^2 of 0.46, p = 0.01(Figure 12)

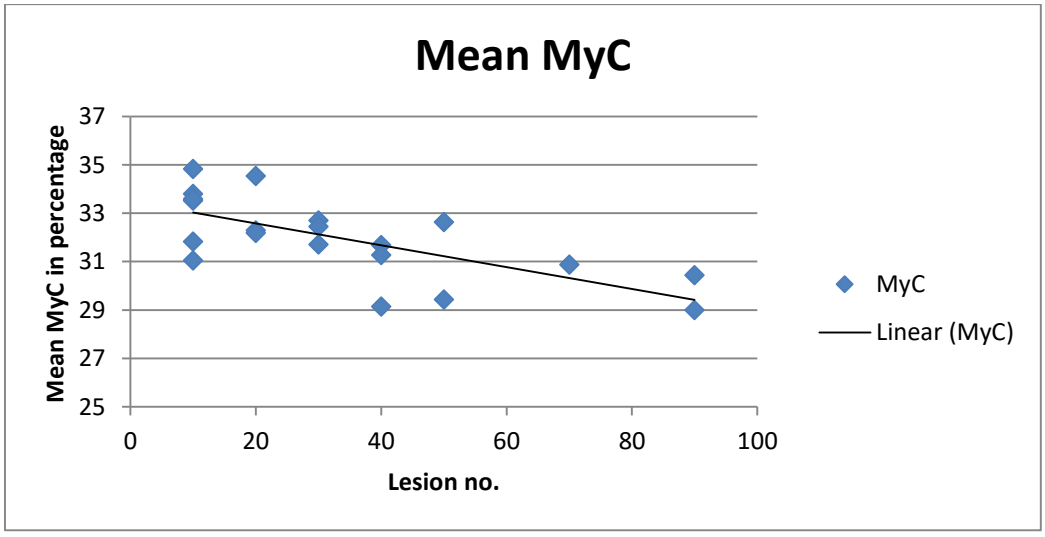

Figure 12 Scatterplot showing correlation of Mean MyC with no. of lesions

6d: Correlation between MyC and EDSS (Expanded disability status scale)

No significant correlation was found between MyC and EDSS. ($p < 0.23$)

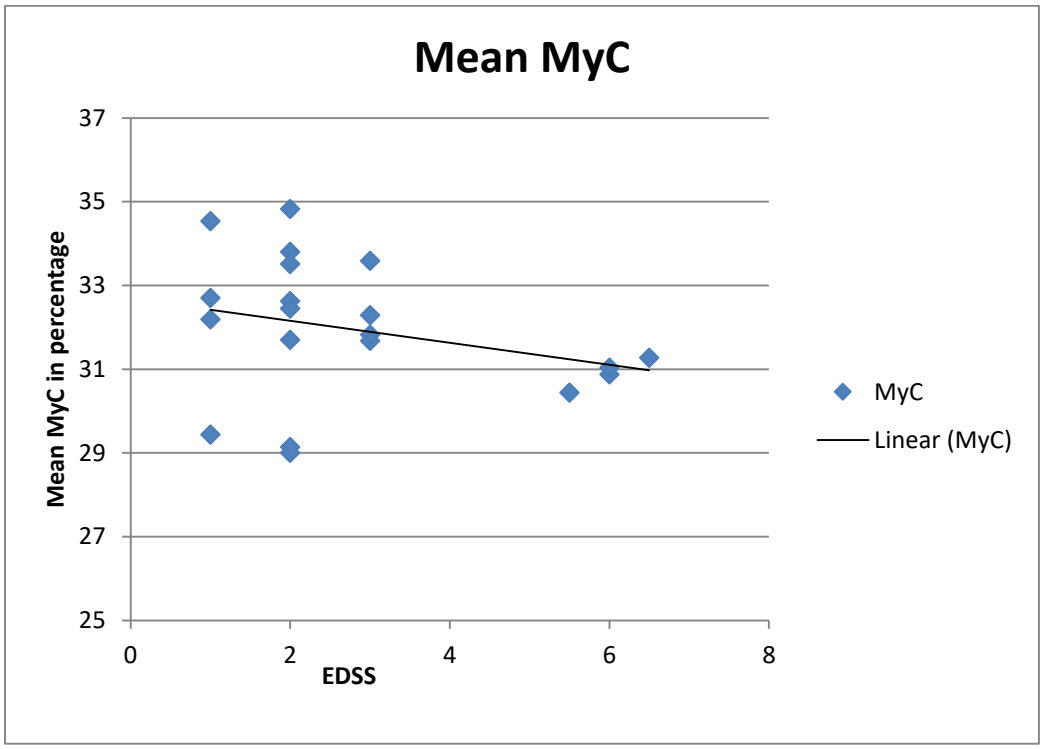

Figure 13 Scatterplot showing correlation of Mean MyC with EDSS

6e: Mean MyC of MS Plaques

The mean Myelin correlated component(MyC) of plaques was 1.72% as compared to NAWM which was 31.95±1.67%. Figure 8 shows comparison of (MyC) in plaques, Normal appearing white matter in patients of MS and Normal white matter in Controls as depicted in Figure 14.

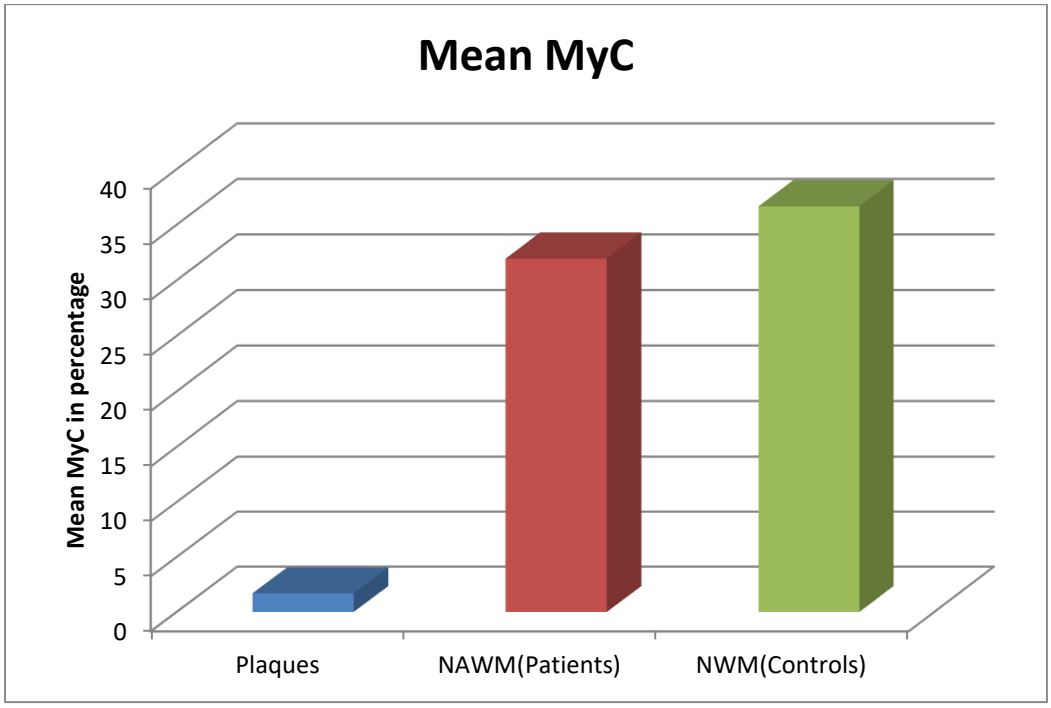

Figure 14 showing comparison of Myelin volume in Plaques, Normal Appearing white matter (NAWM) in patients and Normal white matter(NWM) in controls

7.PERFUSION IMAGING

7a.Quantitative Perfusion measurements (Normalised CBF and CBV)

Normalized CBV(nCBV)

The mean normalized CBV values in patients of MS was 0.40±0.07 as compared to controls which was 0.59±0.07. (Table 8, Figure 15 and Figure 16) with significant p value(<0.05) in all regions of interest.

	Cases (nCBV)		Controls (nCBV)			
	Mean	SD	Mean	SD	t	p-value
R.FRONTAL	0.38	0.09	0.57	0.08	-7.038	0.000
L.FRONTAL	0.38	0.08	0.59	0.09	-7.387	0.000
R.PARIETAL	0.37	0.08	0.56	0.08	-7.581	0.000
L.PARIETAL	0.36	0.09	0.60	0.09	-8.264	0.000
R.TEMPORO-OCCIPITAL	0.36	0.08	0.57	0.06	-9.163	0.000
L.TEMPORO-OCCIPITAL	0.36	0.08	0.59	0.08	-8.308	0.000
GENU	0.47	0.12	0.60	0.13	-3.311	0.002
BODY	0.48	0.12	0.62	0.09	-3.706	0.001
SPLENIUM	0.47	0.12	0.61	0.12	-3.554	0.001
MEAN nCBV	**0.40**	**0.07**	**0.59**	**0.07**	**-7.968**	**0.000**

Table 8 showing normalized CBV in all regions of interest and Mean values in cases and controls.

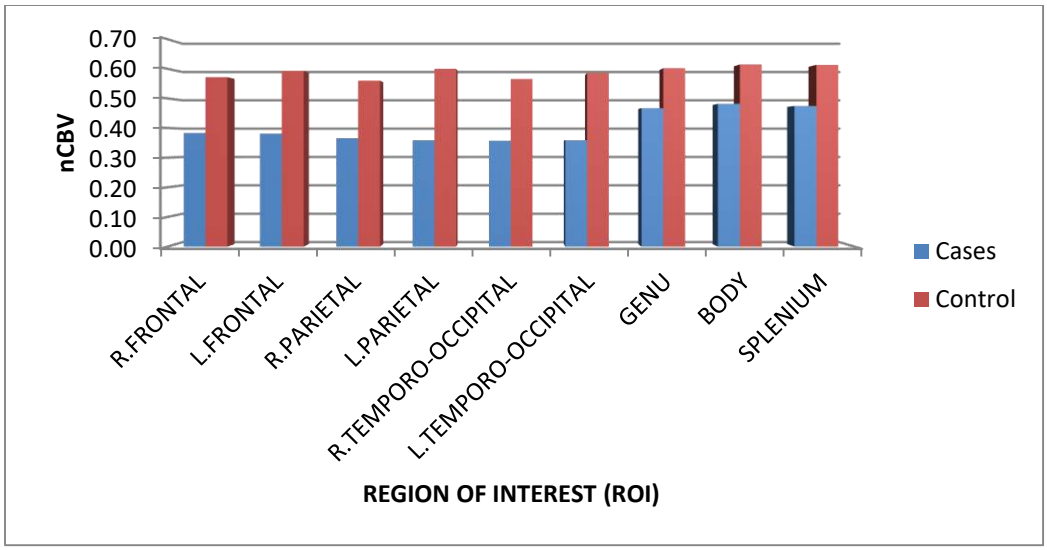

Figure 15 showing comparison of Normalised CBV(nCBV) in all regions of interest in cases and controls

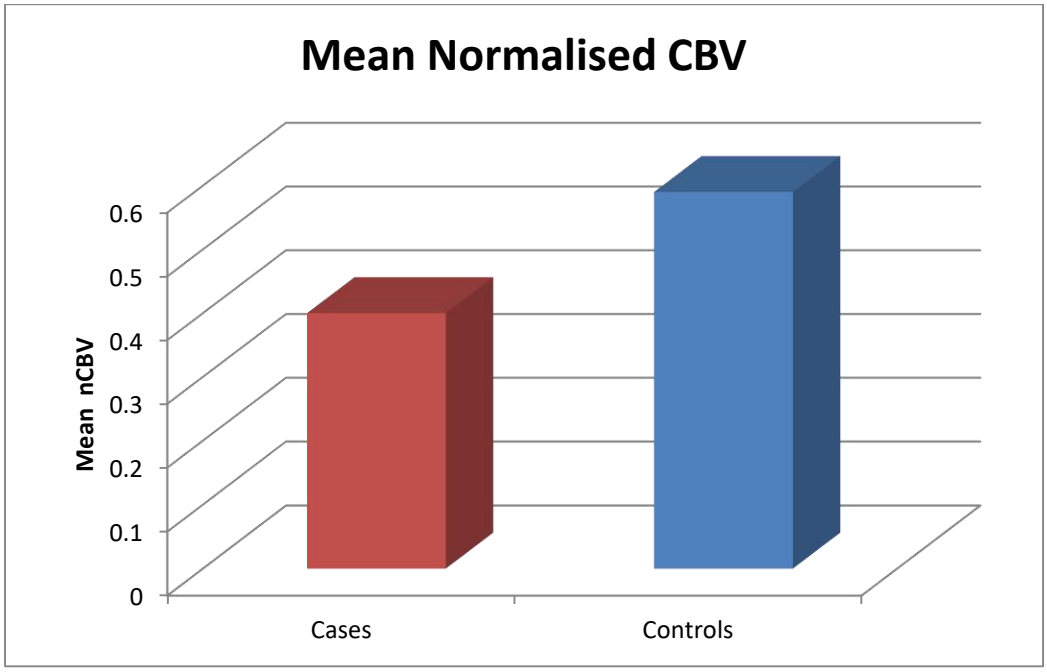

Figure 16 showing comparison for mean normalized CBV of cases and controls

Normalised CBF(nCBF)

The mean normalized CBF value of NAWM in patients with MS was 0.40±0.07 as compared to controls which was 0.59±0.07 ($p < 0.05$). Table 9 and Figure 17 show regional normalised CBF in NAWM in patients with MS and in healthy controls. Figure 18 shows mean normalized CBF in NAWM in cases and controls.

	Cases (nCBF)		Control (nCBF)			
	Mean	SD	Mean	SD	t	p-value
R.FRONTAL	0.37	0.07	0.53	0.08	-6.645	0.000
L.FRONTAL	0.40	0.07	0.55	0.08	-6.209	0.000
R.PARIETAL	0.37	0.06	0.56	0.10	-7.024	0.000
L.PARIETAL	0.36	0.07	0.56	0.10	-6.891	0.000
R.TEMPORO-OCCIPITAL	0.37	0.07	0.55	0.09	-7.206	0.000
L.TEMPORO-OCCIPITAL	0.39	0.07	0.55	0.07	-6.937	0.000
GENU	0.46	0.10	0.58	0.10	-3.401	0.002
BODY	0.45	0.10	0.57	0.11	-3.639	0.001
SPLENIUM	0.46	0.11	0.59	0.10	-3.688	0.001
MEAN nCBF	**0.40**	**0.06**	**0.55**	**0.07**	**-7.274**	**0.000**

Table 9 shows nCBF in all regions of interest in NAWM in MS patients and controls.

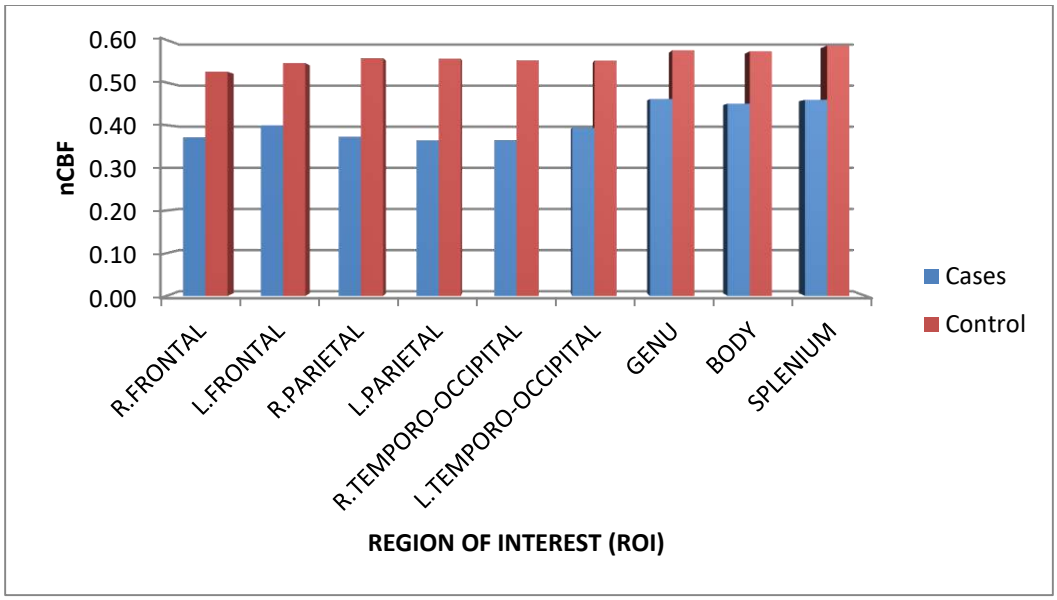

Figure 17 showing comparison of Normalised CBF in all regions of interest in cases and controls

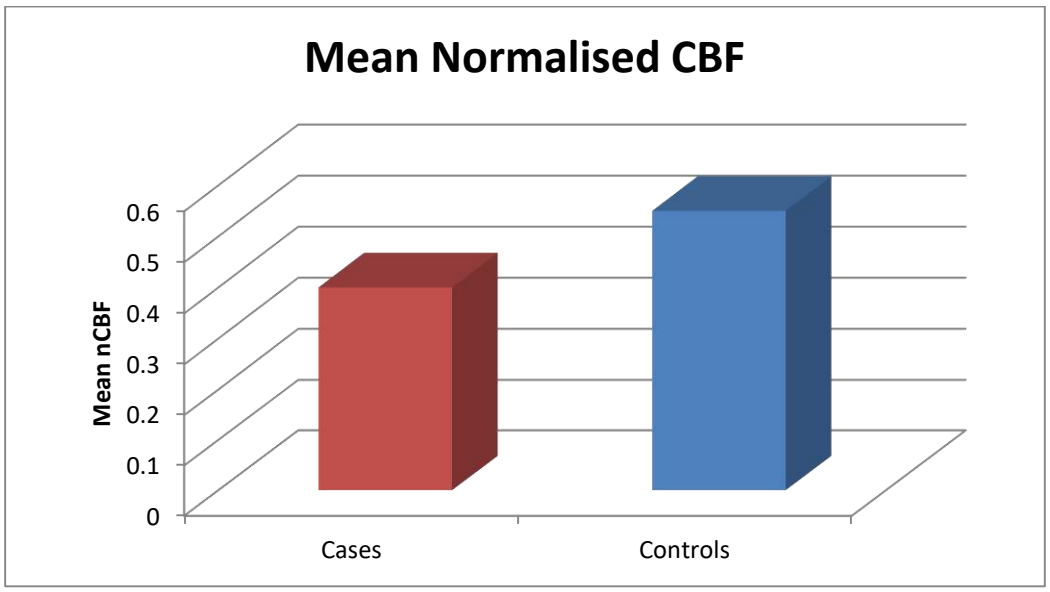

Figure 18 showing comparison of Mean normalized CBF of NAWM in cases and controls

7b:Correlation of nCBV and nCBF with lesion load, disease duration and EDSS

No significant correlation was found between nCBF, nCBV with disease duration, EDSS or lesion load.

7c:Correlation between CBF and CBV

Significant correlation was found between CBV and CBF with Pearson correlation of 0.514, r^2= 0.26 (p=0.020) as shown in Figure 19.

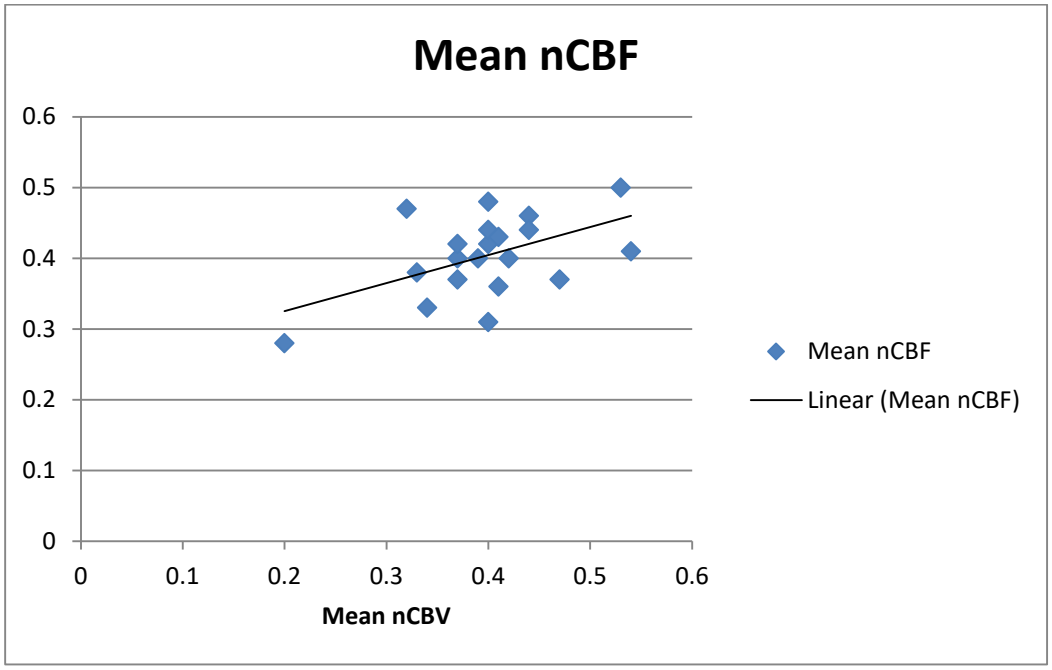

Figure 19 showing scatterplot chart of correlation between normalized CBV and normalized CBF

OBSERVATIONS

1. MYELIN IMAGING

Control 1

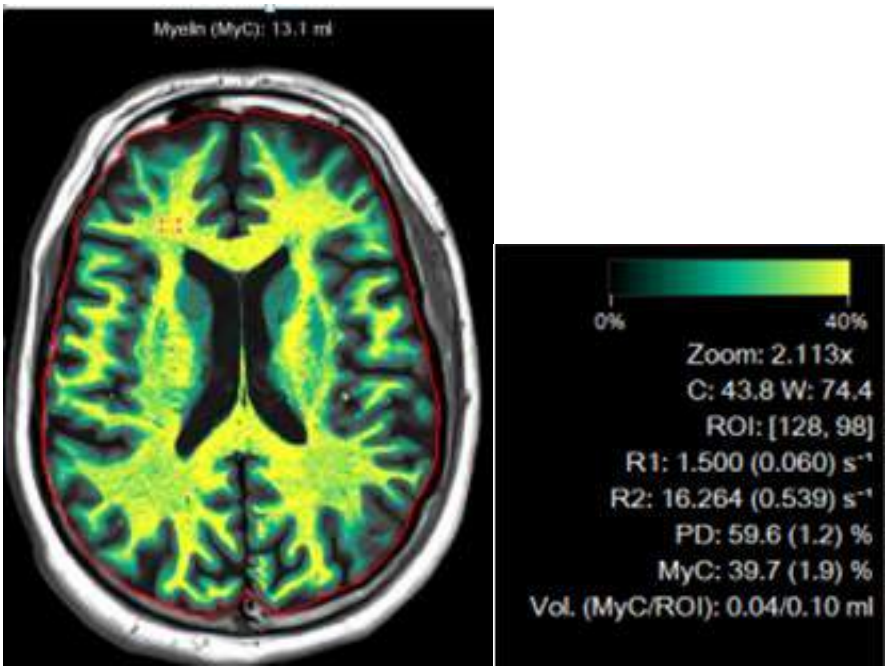

Figure 20a Control 1 with ROI in right frontal region(MyC= 39.7±1.9%)

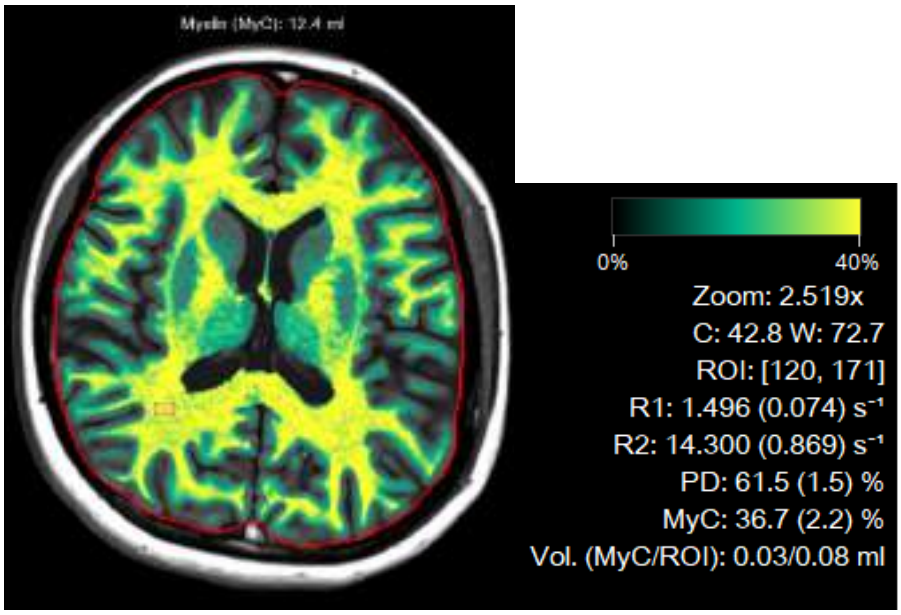

Figure 20b: Control 1 with ROI in left frontal region (MyC=36.7±2.2%)

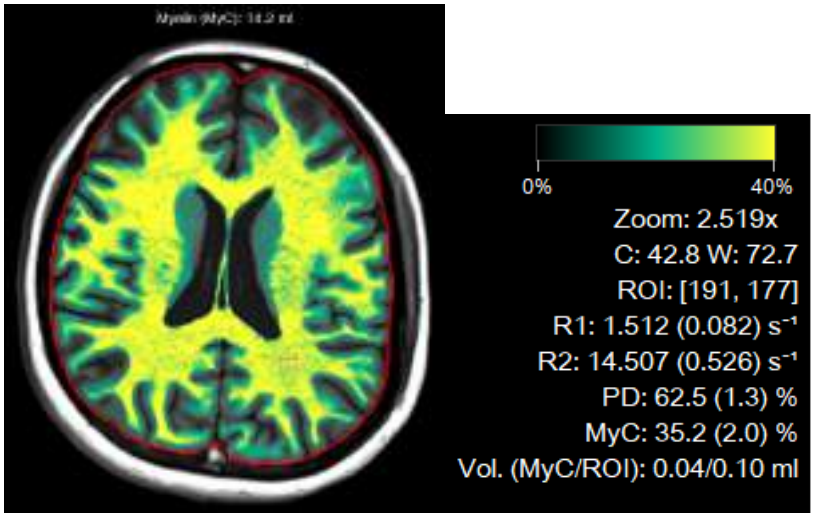

Figure 20c: Control 1 with ROI in left parietal region (MyC=35.2±2%)

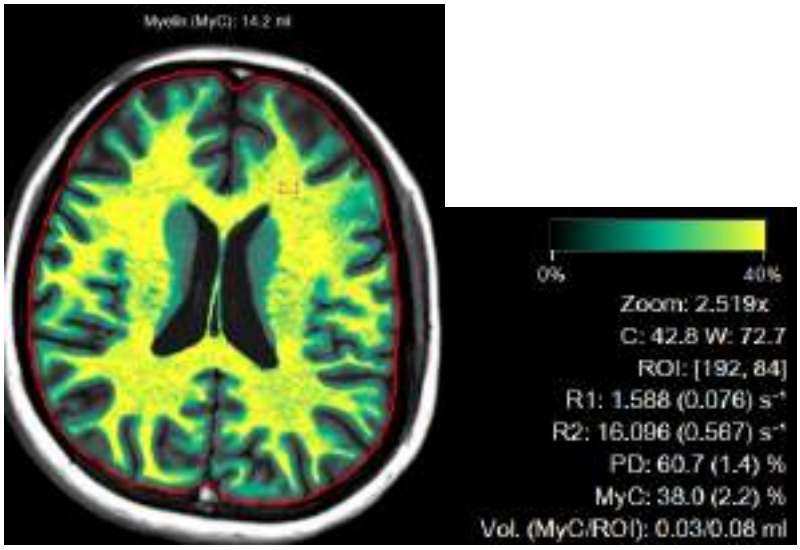

Figure 20d: Control 1 with ROI in left parietal region (MyC=38±2.2%)

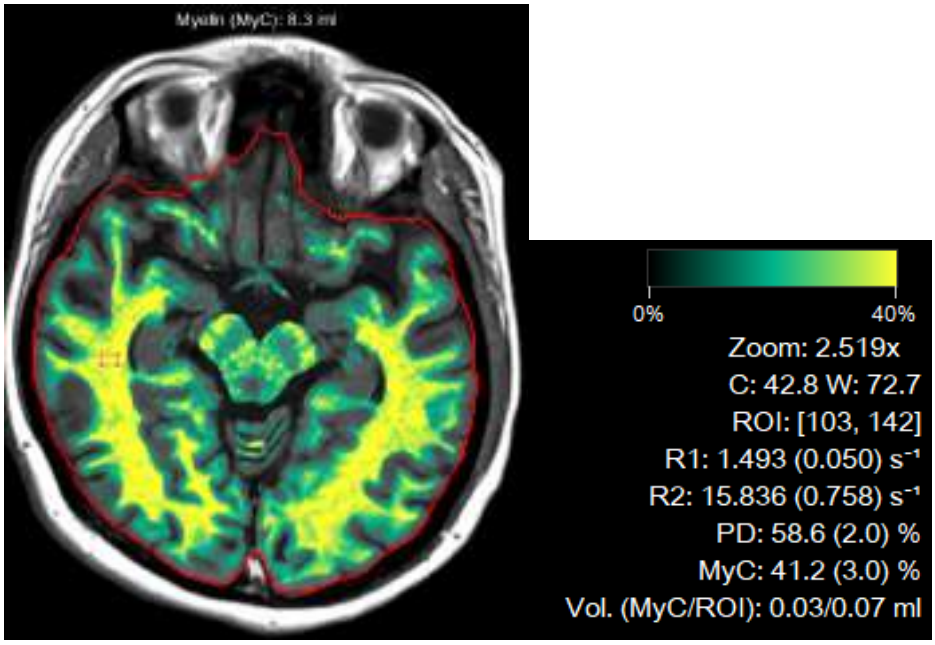

Figure 20e: Control 1 with ROI in right temporo-occipital region (MyC=41.2±3%)

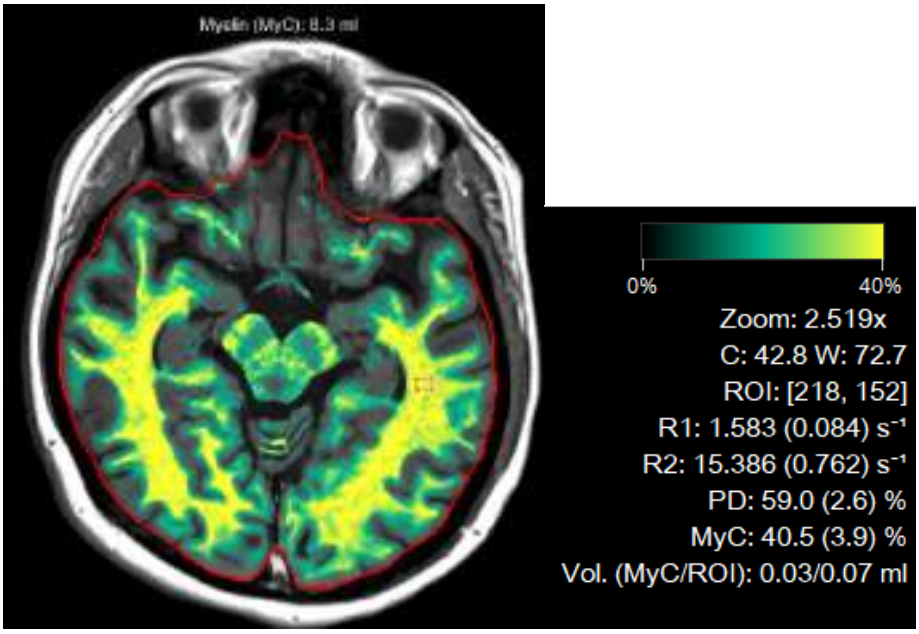

Figure 20f: Control 1 with ROI in left temporo-occipital region (MyC=40.5±3.9%)

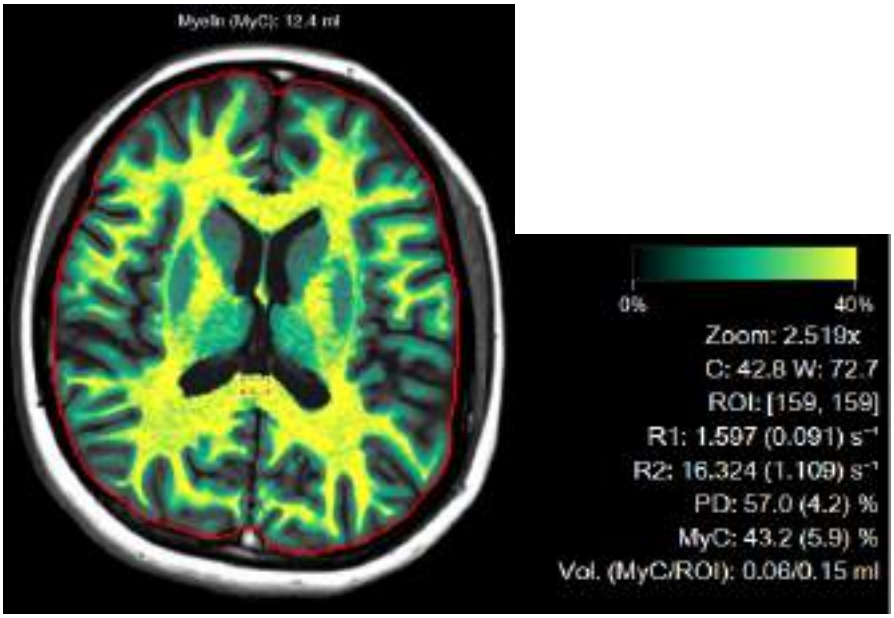

Figure 20g: Control 1 with ROI in splenium (MyC=43.2±5.9%)

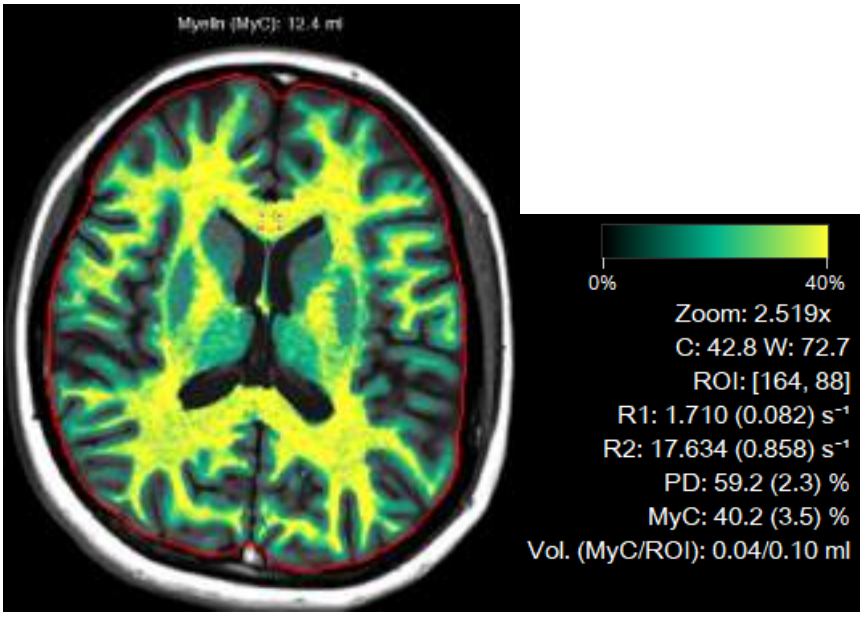

Figure 20h: Control 1 with ROI in genu (MyC=40.2±3.5%)

Case 1

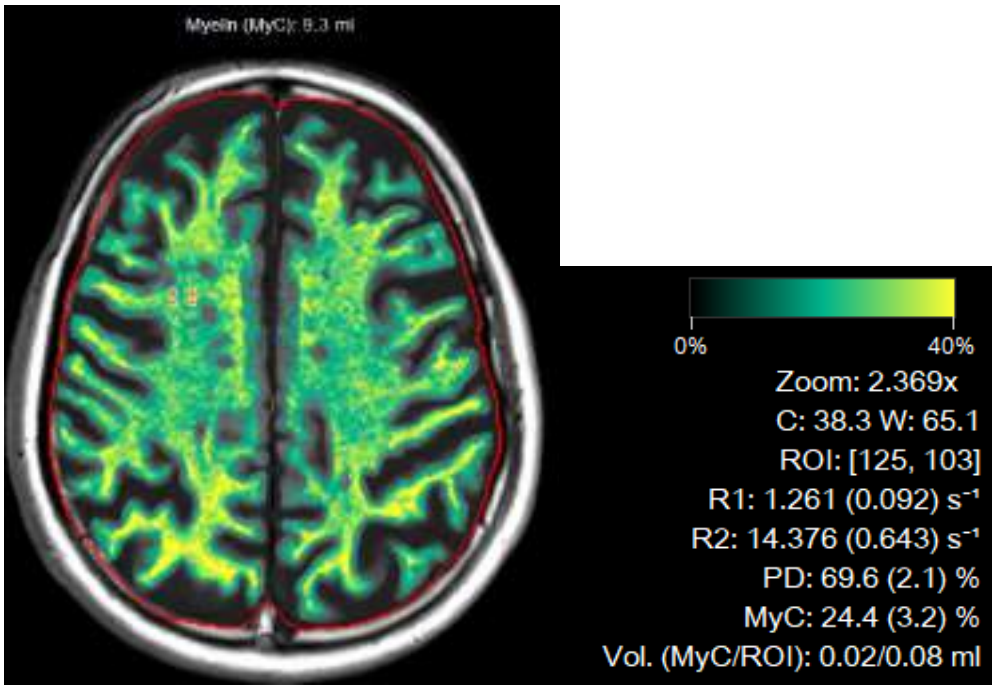

Figure 21a: Case 1 with ROI in NAWM in right frontal region (MyC=24.4±3.2%)

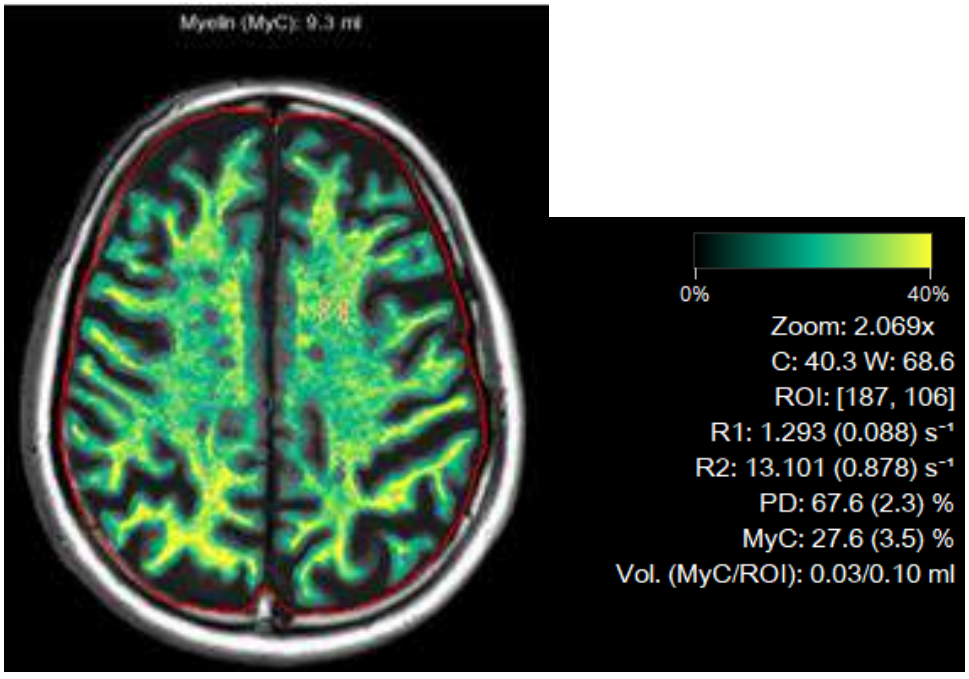

Figure 21b: Case 1 with ROI in NAWM in left frontal region (MyC=27.6±3.5%)

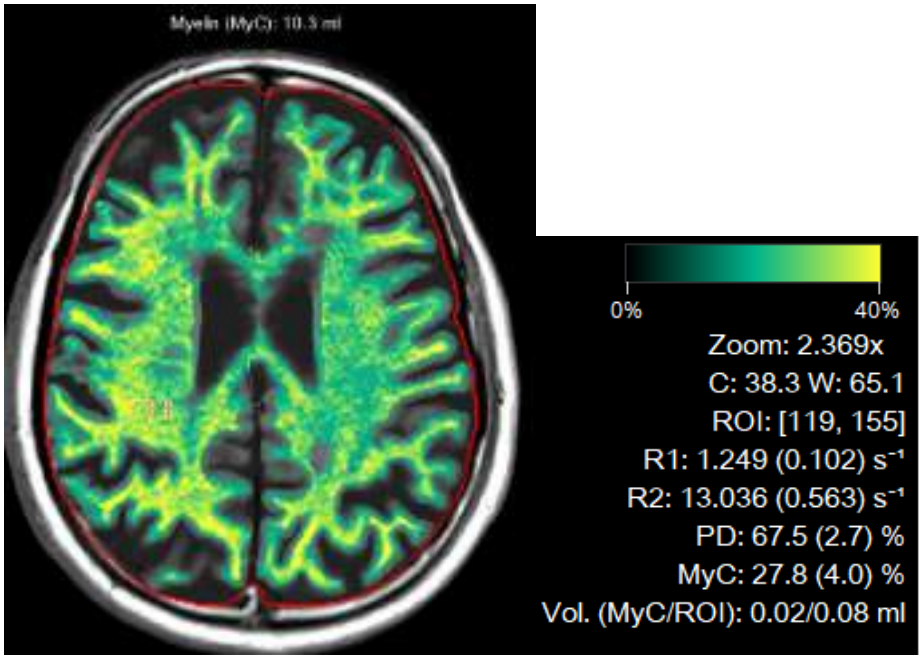

Figure 21c: Case 1 with ROI in nawm in right parietal region (MyC=27.8±4.0%)

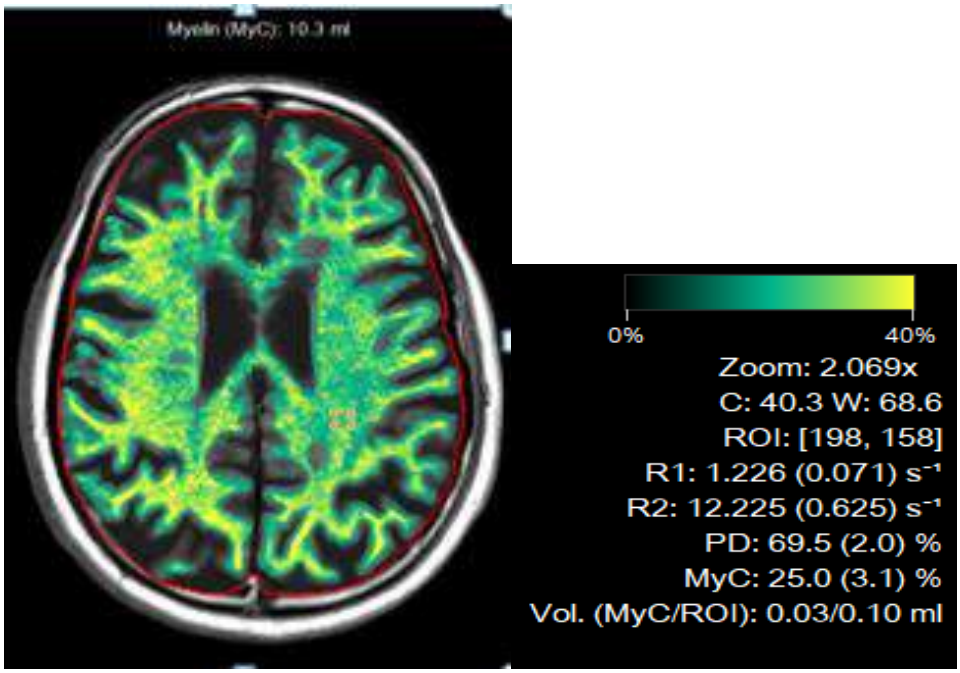

Figure 21d: Case 1 with ROI in NAWM in left parietal region (MyC=25±3.1%)

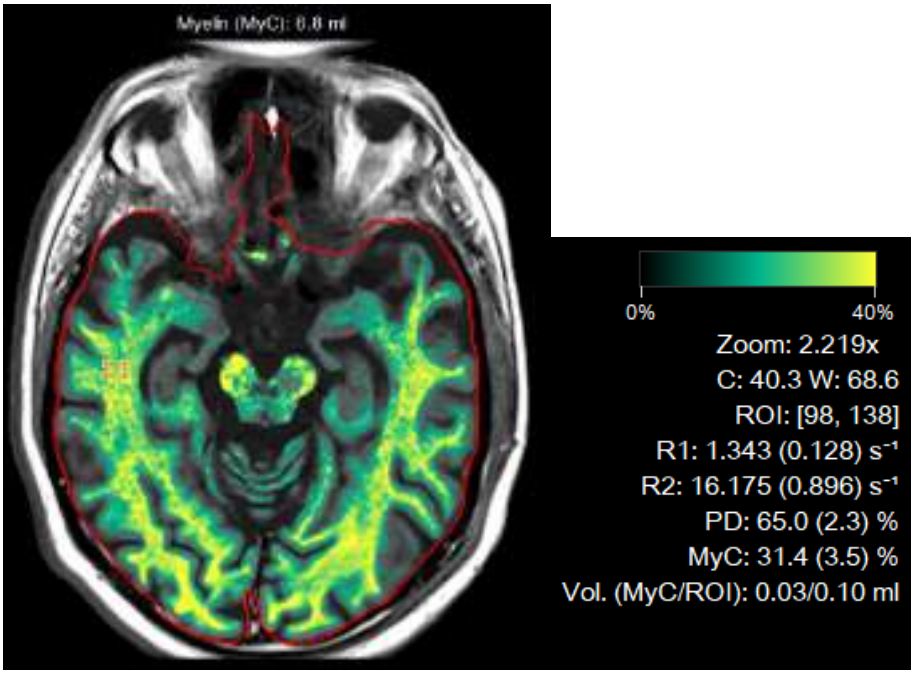

Figure 21e: Case 1 with ROI in NAWM in right temporo-occipital region (MyC=31.4±3.5%)

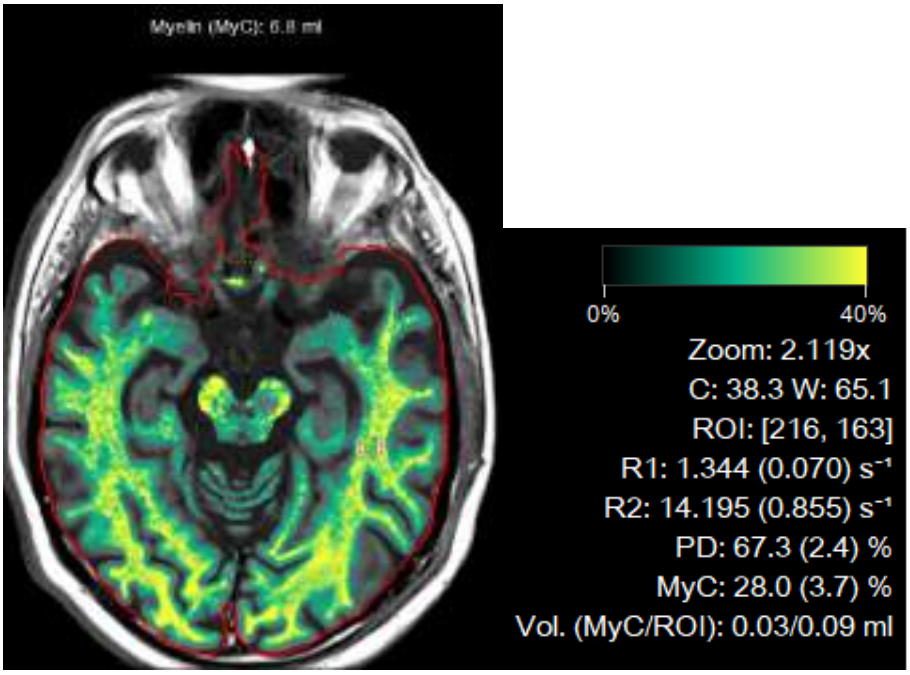

Figure 21f: Case 1 with ROI in NAWM in left temporo-occipital region (MyC=28±3.7%)

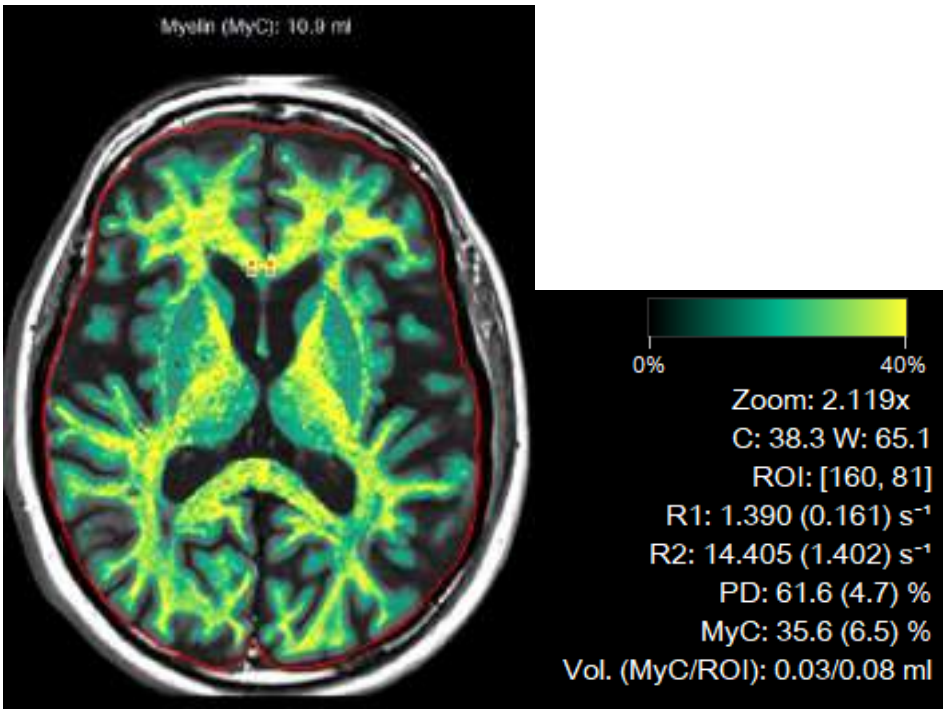

Figure 21g: Case 1 with ROI in genu of corpus callosum(MyC=35.6±6.5%)

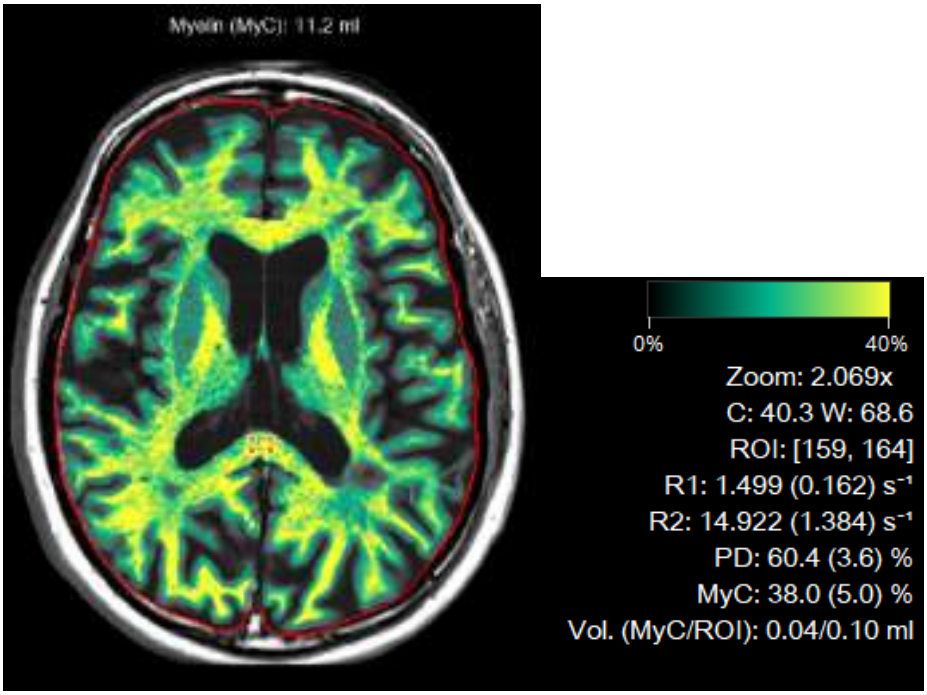

Figure 21h: Case 1 with ROI in splenium of corpus callosum (MyC=38±5.0%)

Case 2

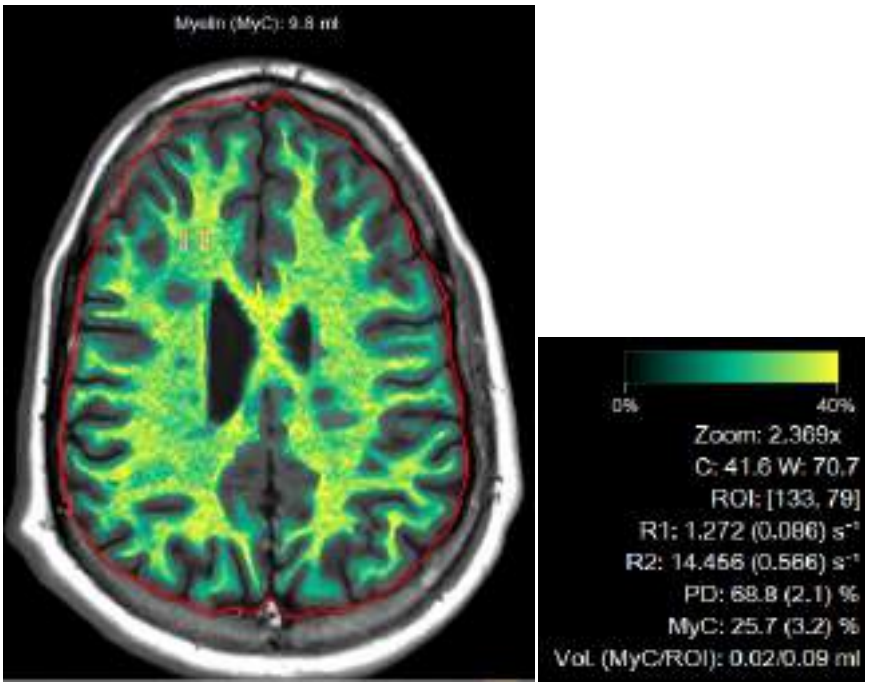

Figure 22a: Case 2 with ROI in NAWM in right frontal region (MyC=25.7±3.2%)

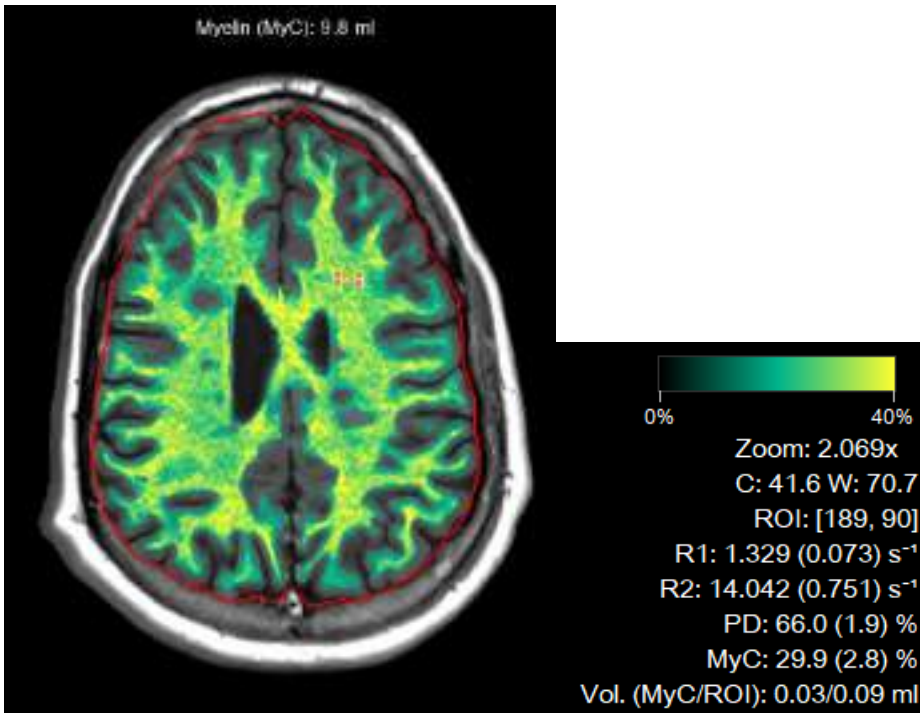

Figure 22b: Case 2 with ROI in NAWM in left frontal region (MyC=29.9±2.8%)

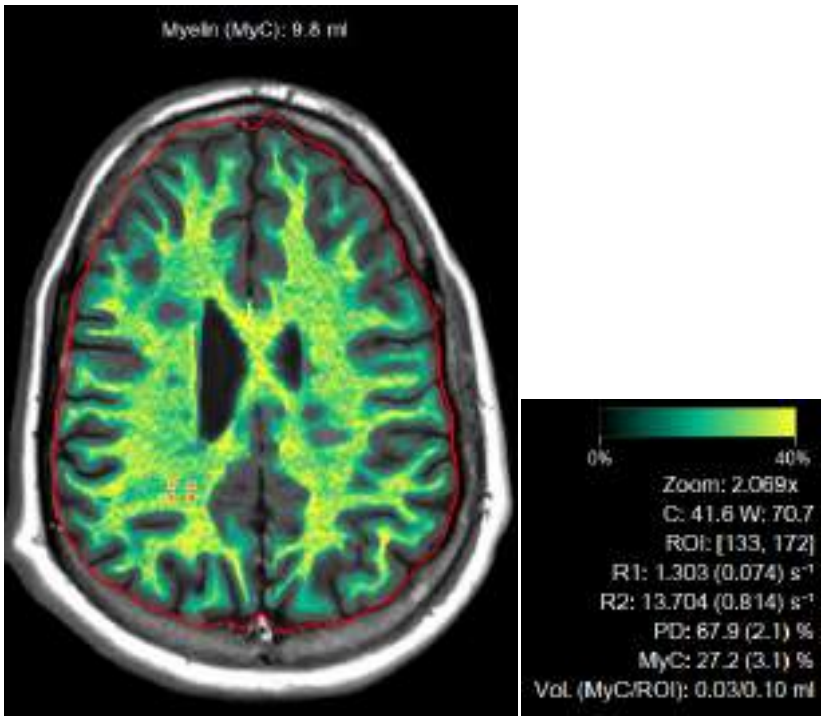

Figure 22c: Case 2 with ROI in NAWM in right parietal region (MyC=27.2±3.1%)

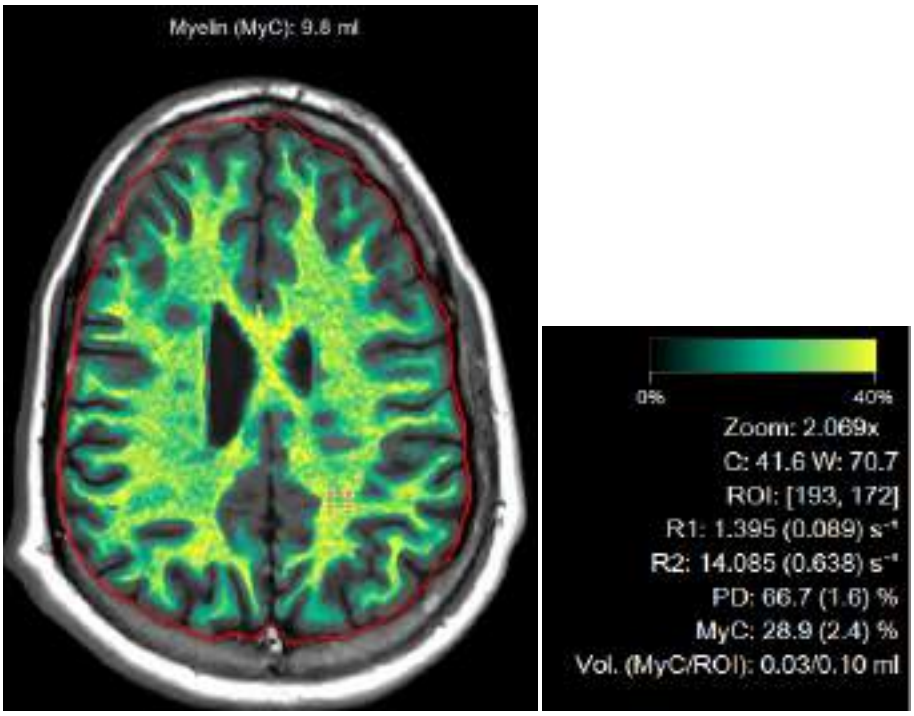

Figure 22d: Case 2 with ROI in NAWM in left parietal region (MyC=28.9±2.4%)

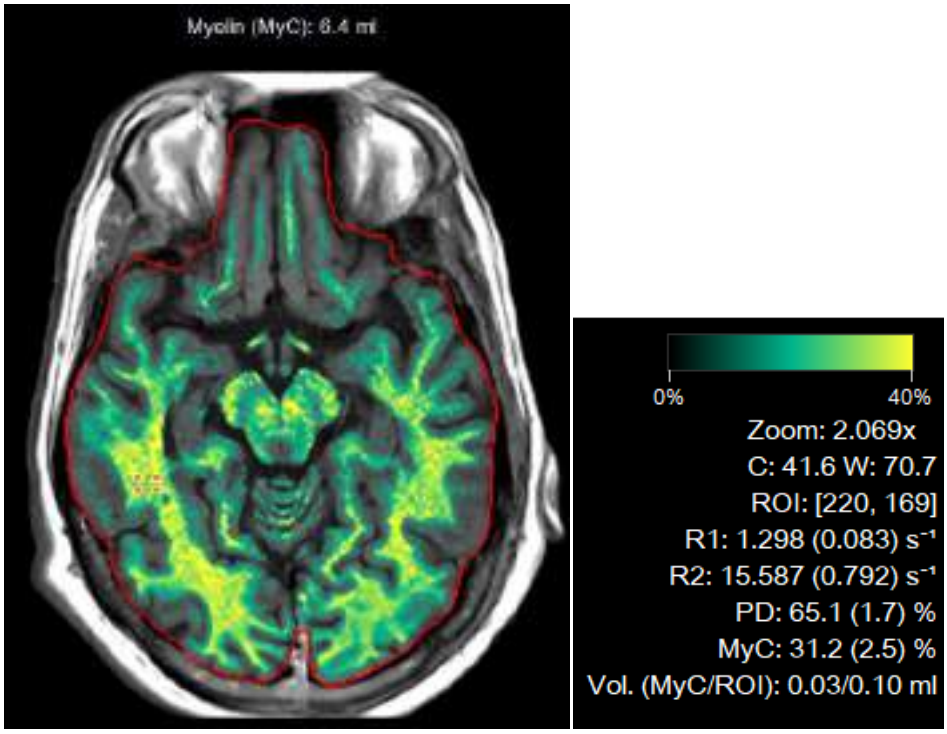

Figure 22e: Case 2 with ROI in NAWM in right temporo-occipital region (MyC=31.2±2.5%)

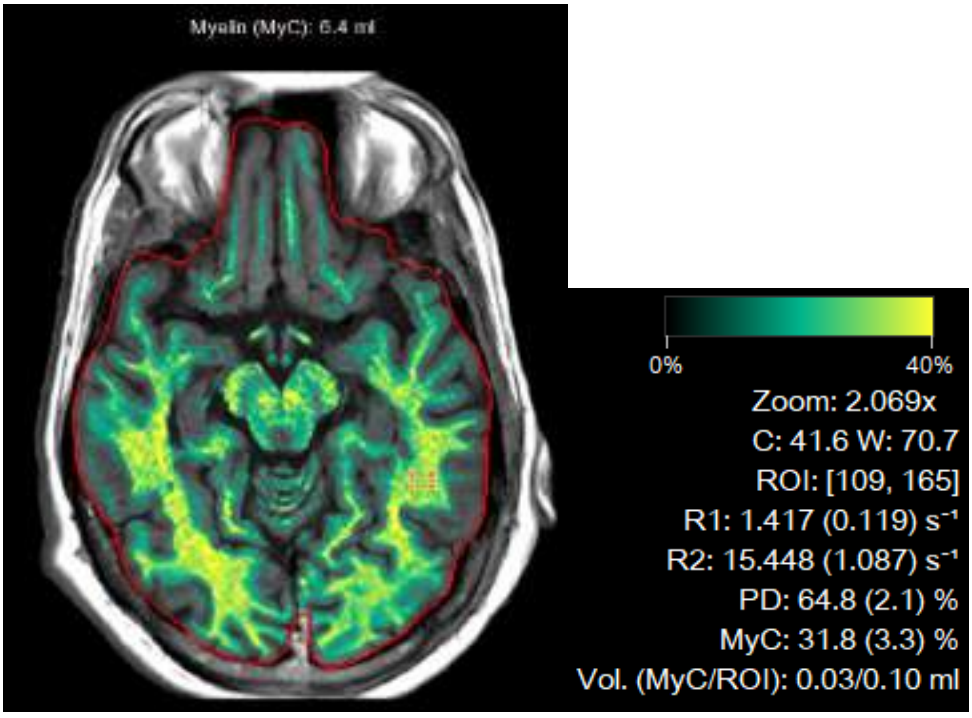

Figure 22f: Case 2 with ROI in NAWM in left temporo-occipital region (MyC=31.8±3.3%)

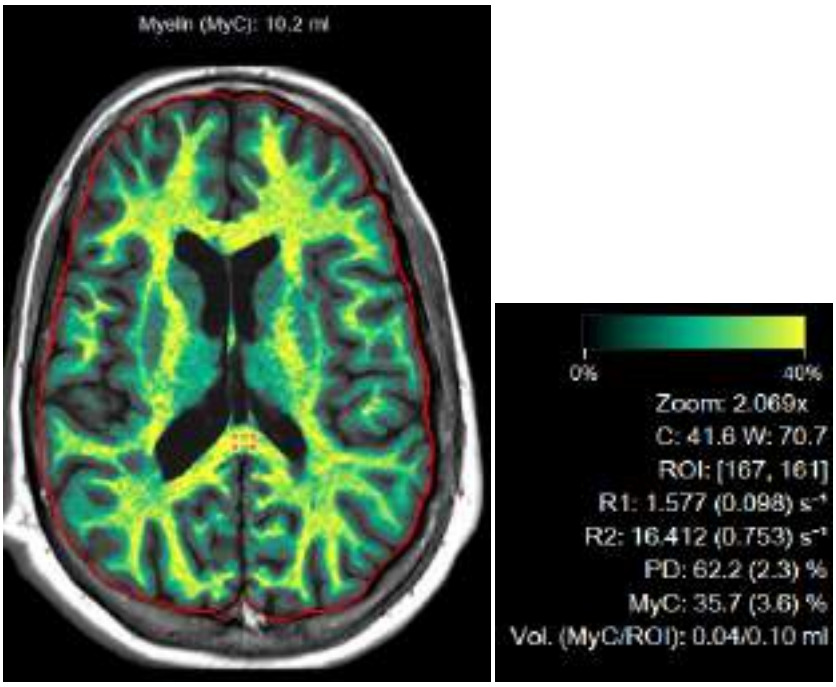

Figure 22g: Case 2 with ROI in splenium (MyC=35.7±3.6%)

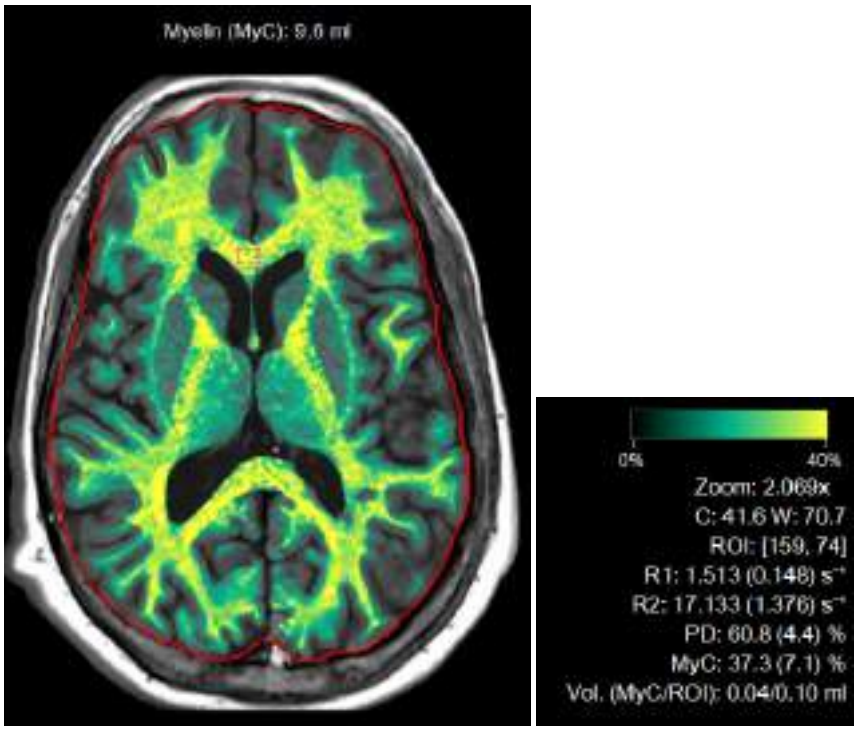

Figure 22h: Case 2 with ROI in genu (MyC=37.3±7.1%)

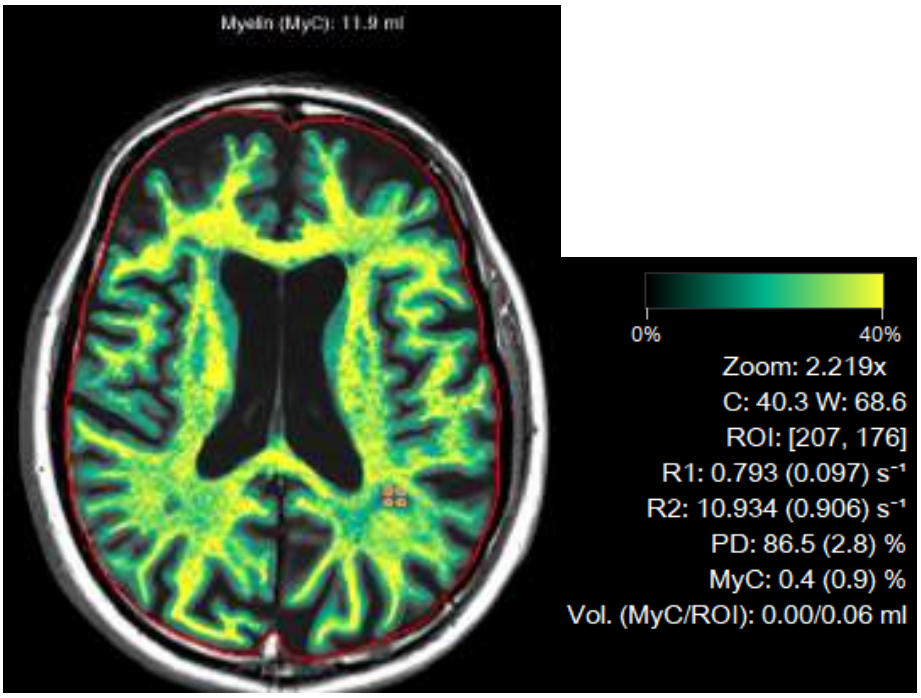

Figure 23a: ROI Placement in MS Plaque in periventricular white matter in left parietal region

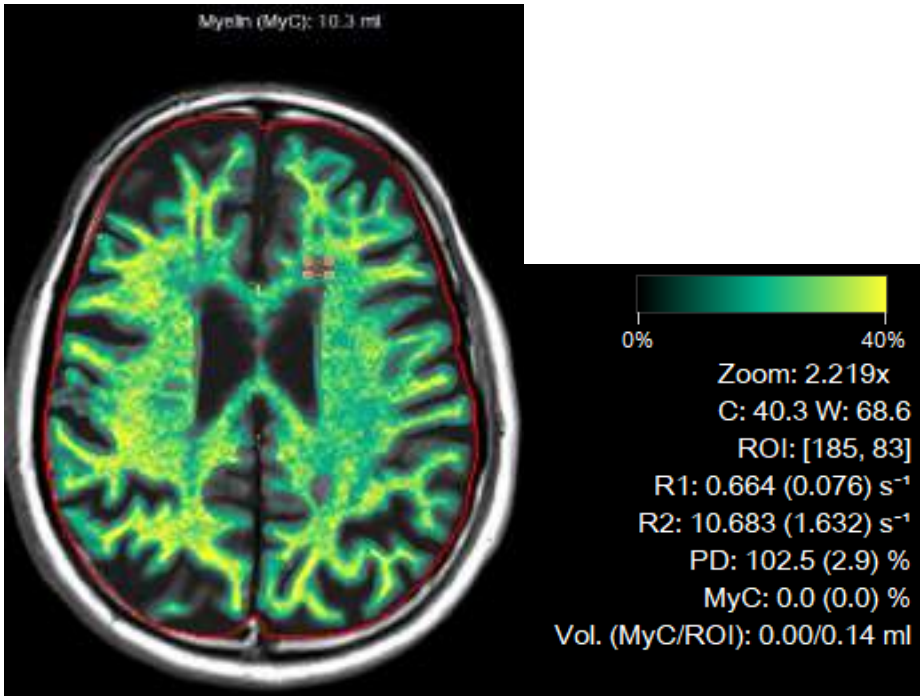

Figure 23b:ROI Placement in MS Plaque in periventricular white matter in left frontal region

2. PERFUSION IMAGING

Control 1

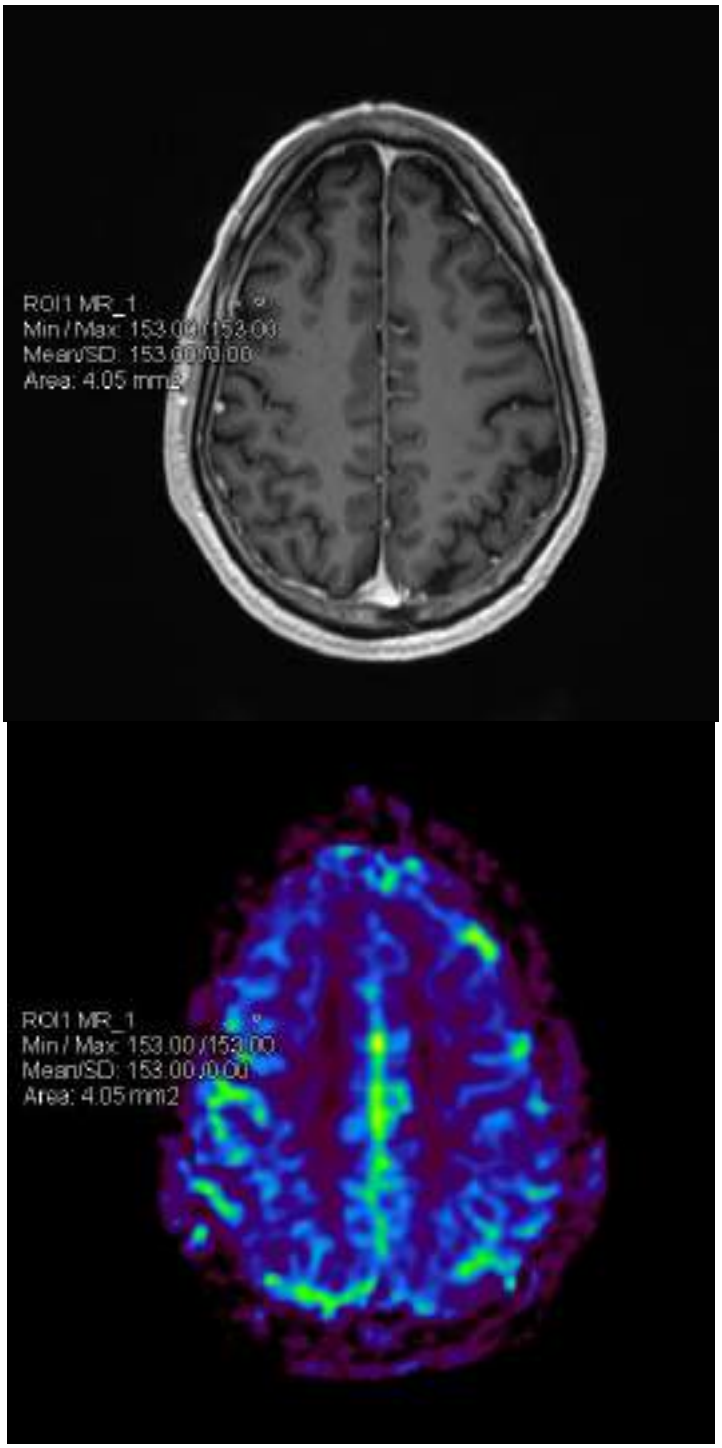

Figure 24a:Control 1 with ROI at grey matter right used for normalisation in right frontal region= 153

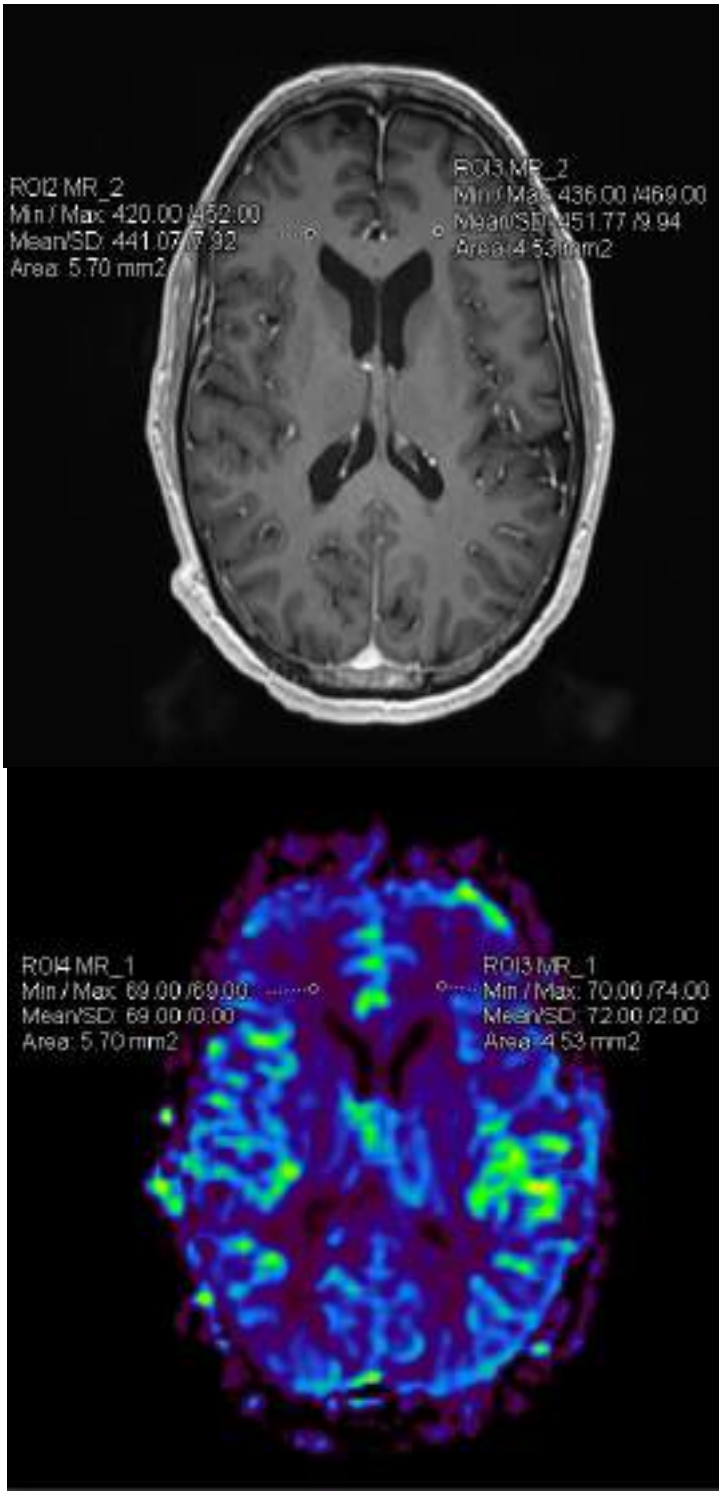

Figure 24b: Control 1 with normalised CBF Right and left frontal region is 0.45 and 0.47 respectively

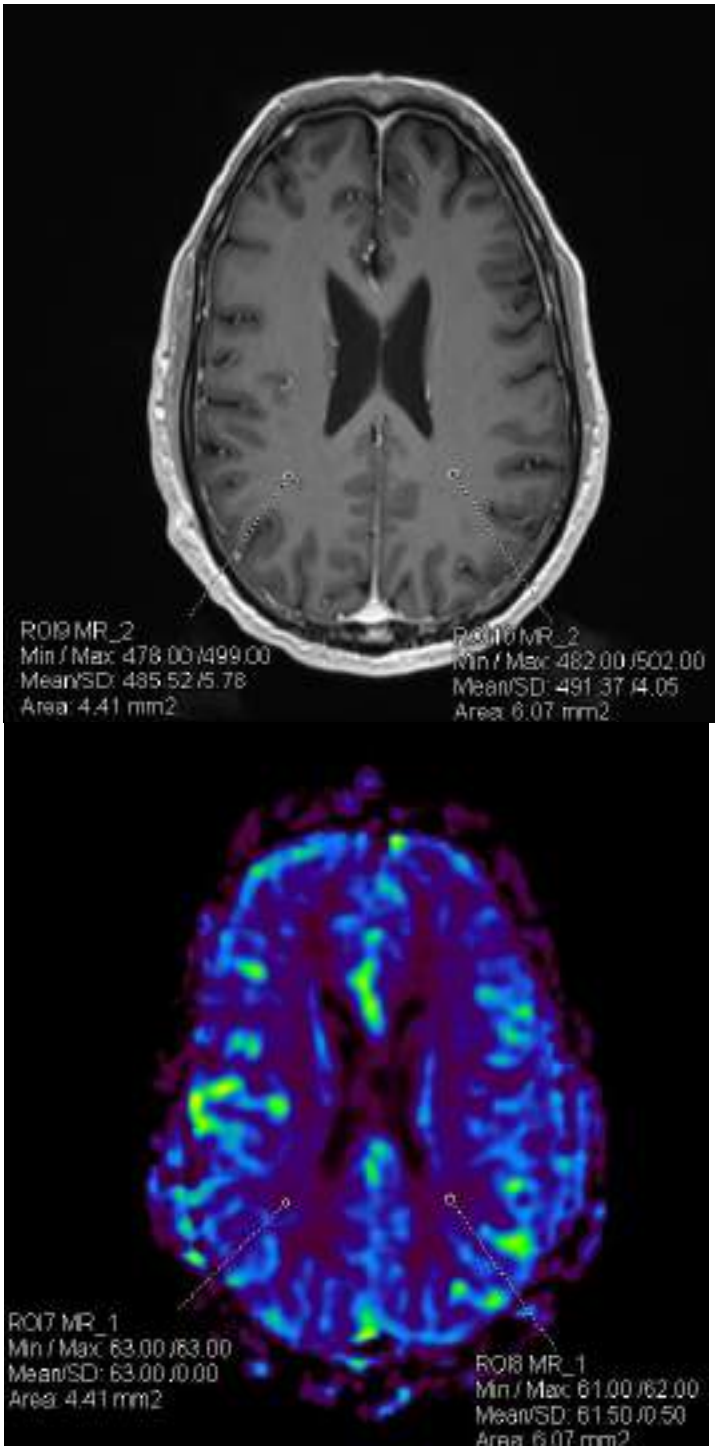

Figure 24c: Control 1 with normalised CBF Right and left parietal region is 0.41 and 0.40 respectively

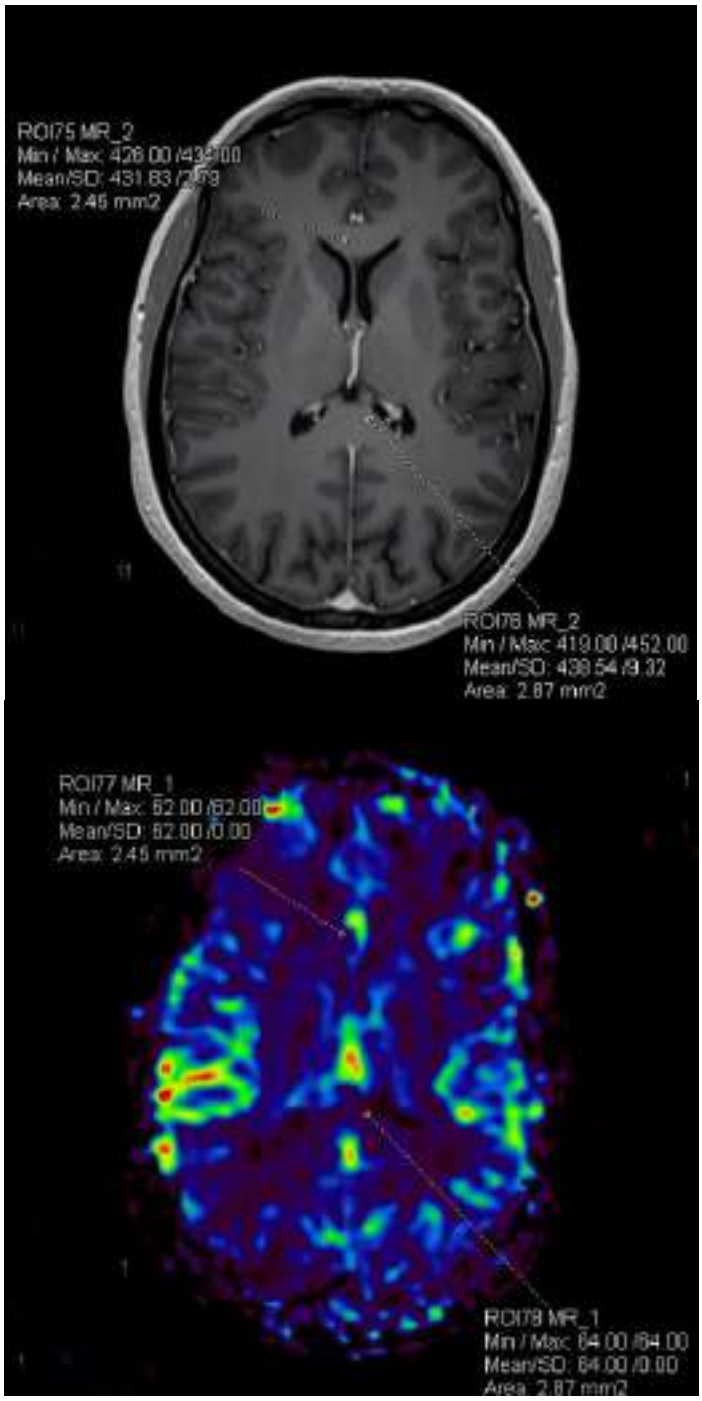

Figure 24d: Control 1 with normalised CBF of genu and splenium is 0.40 and 0.41 respectively

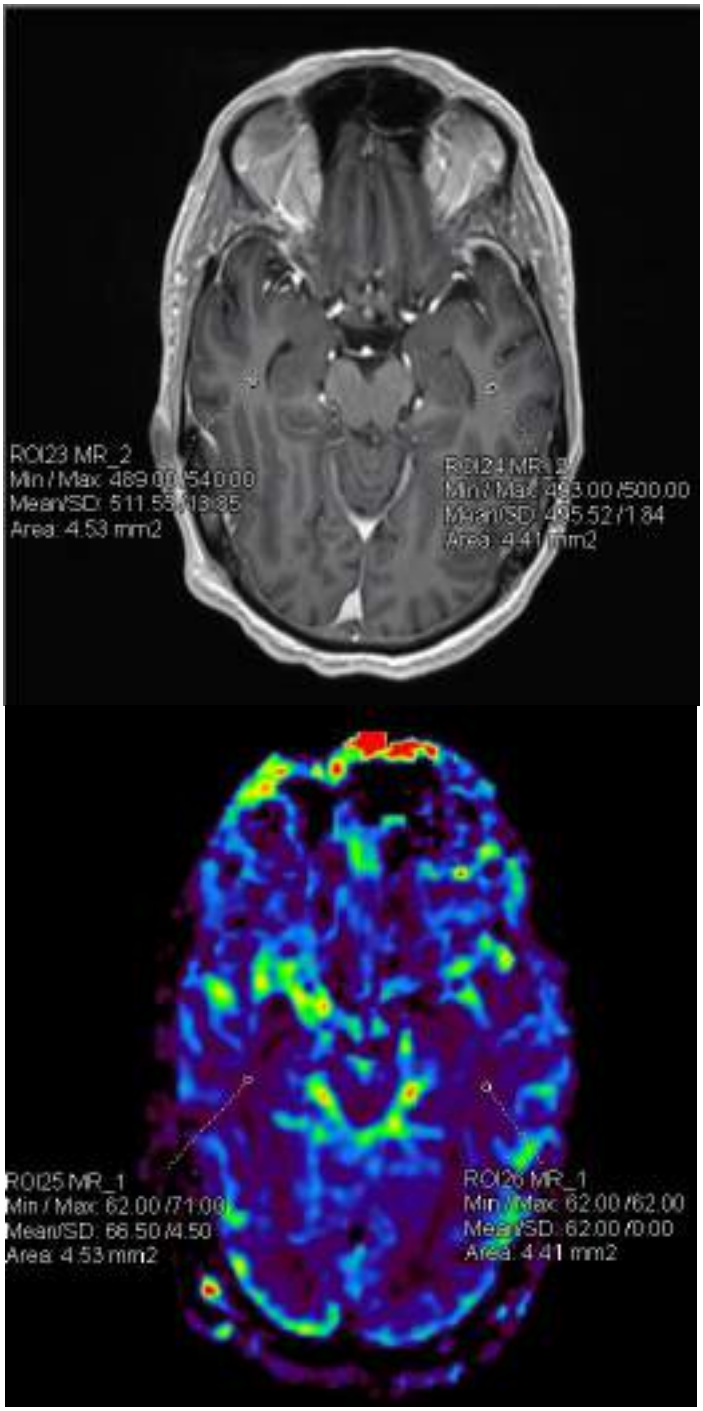

Figure 24e: Control 1 with normalised CBF Right and left temporo-occipitalregion is 0.43 and 0.40 respectively

CASE 1

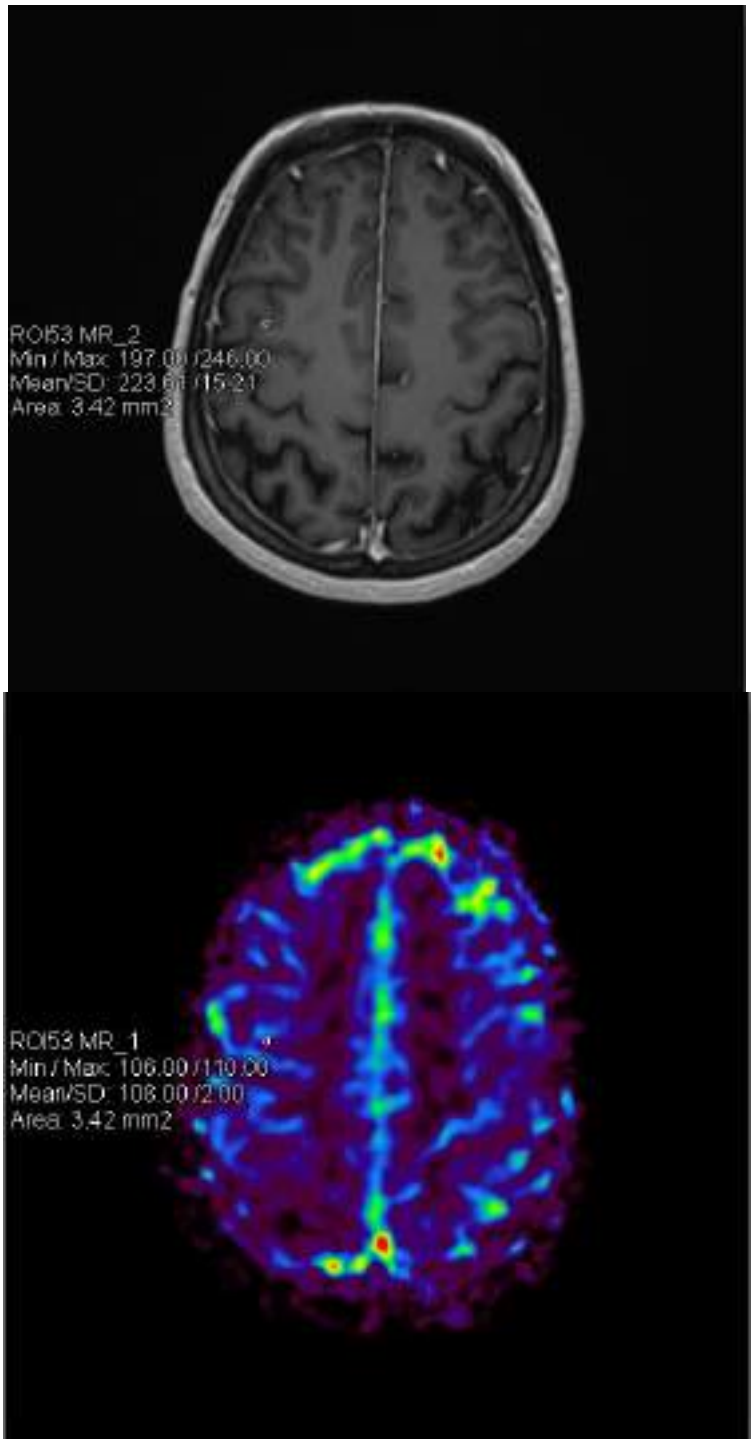

Figure 25a: Case 1 with ROI at grey matter right frontal region used for normalisation= 108

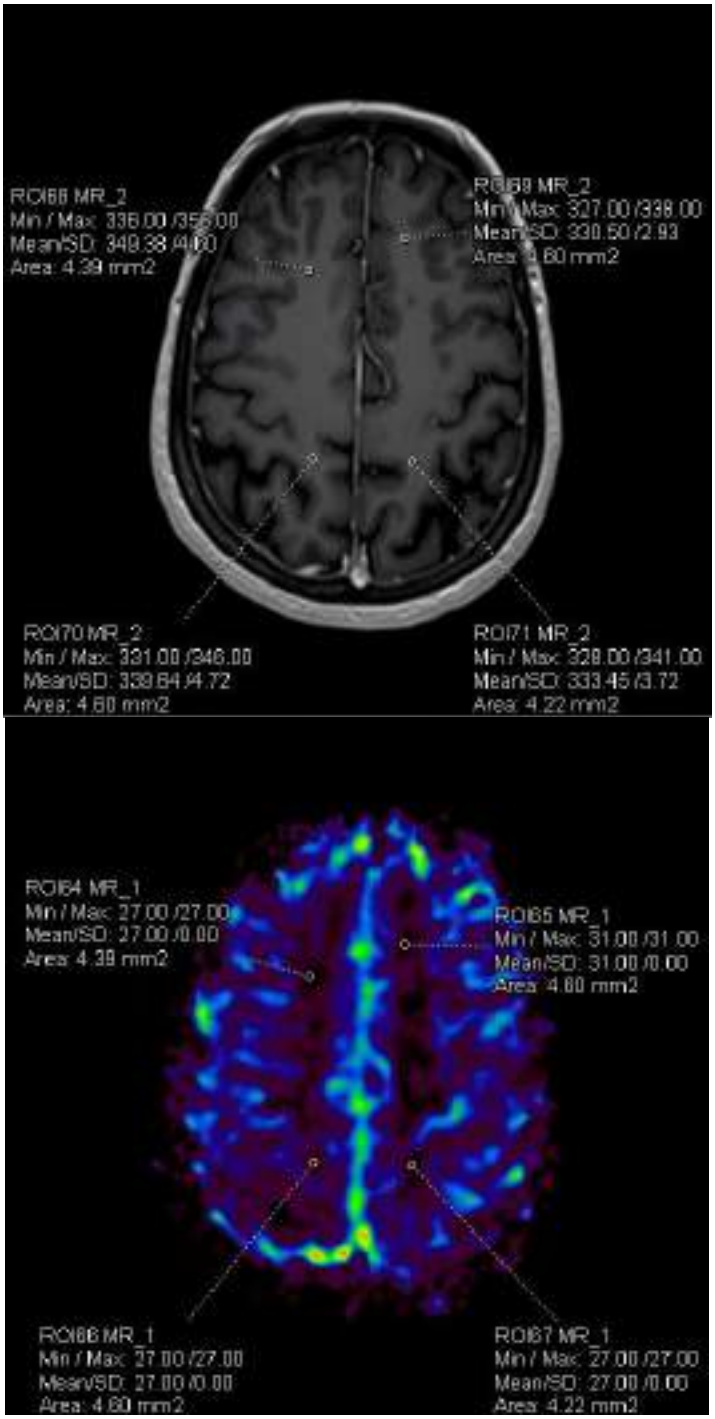

Figure 25b: Case 1: normalised CBV for right and left frontal region is 0.25 and 0.28 respectively

Normalised CBV for right and left parietal region is 0.25 and 0.25 respectively

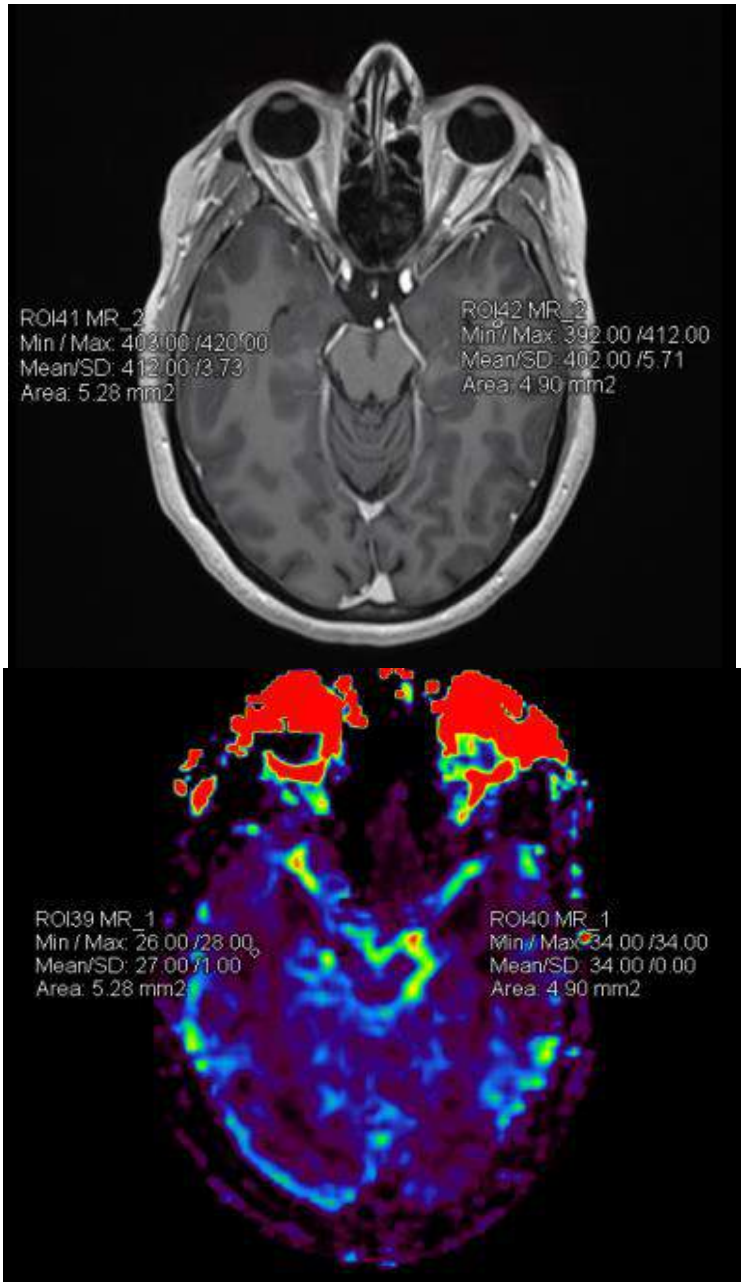

Figure 25c: Case 1 with normalised CBV For right and left temporo-occipital regions is 0.25 and 0.31 respectively

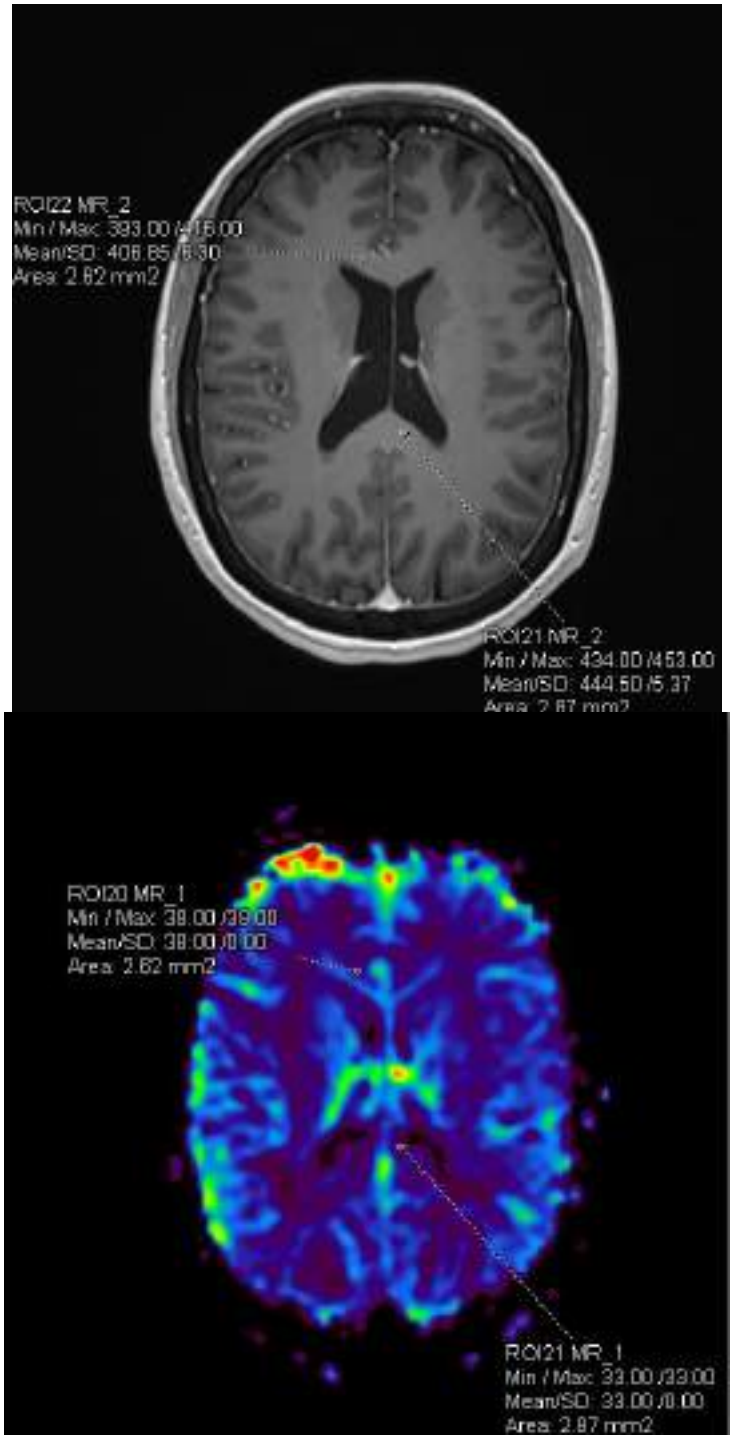

Figure 25d: Case 1 with normalised CBV of genu and splenium and respectively 0.35 and 0.30 respectively

CASE 2

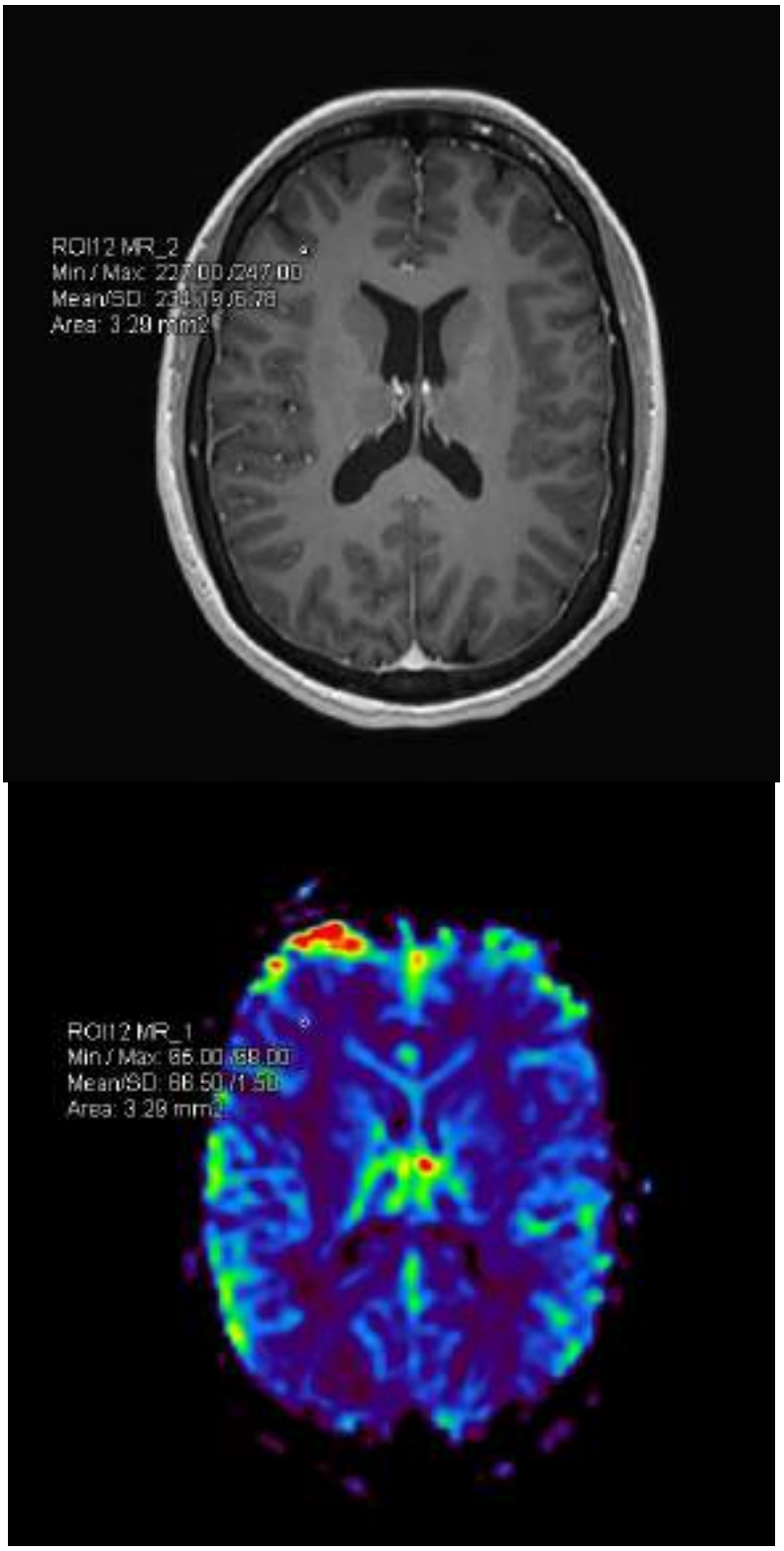

Figure 26a Case 2 with ROI at of grey matter right frontal region used for normalisation = 66

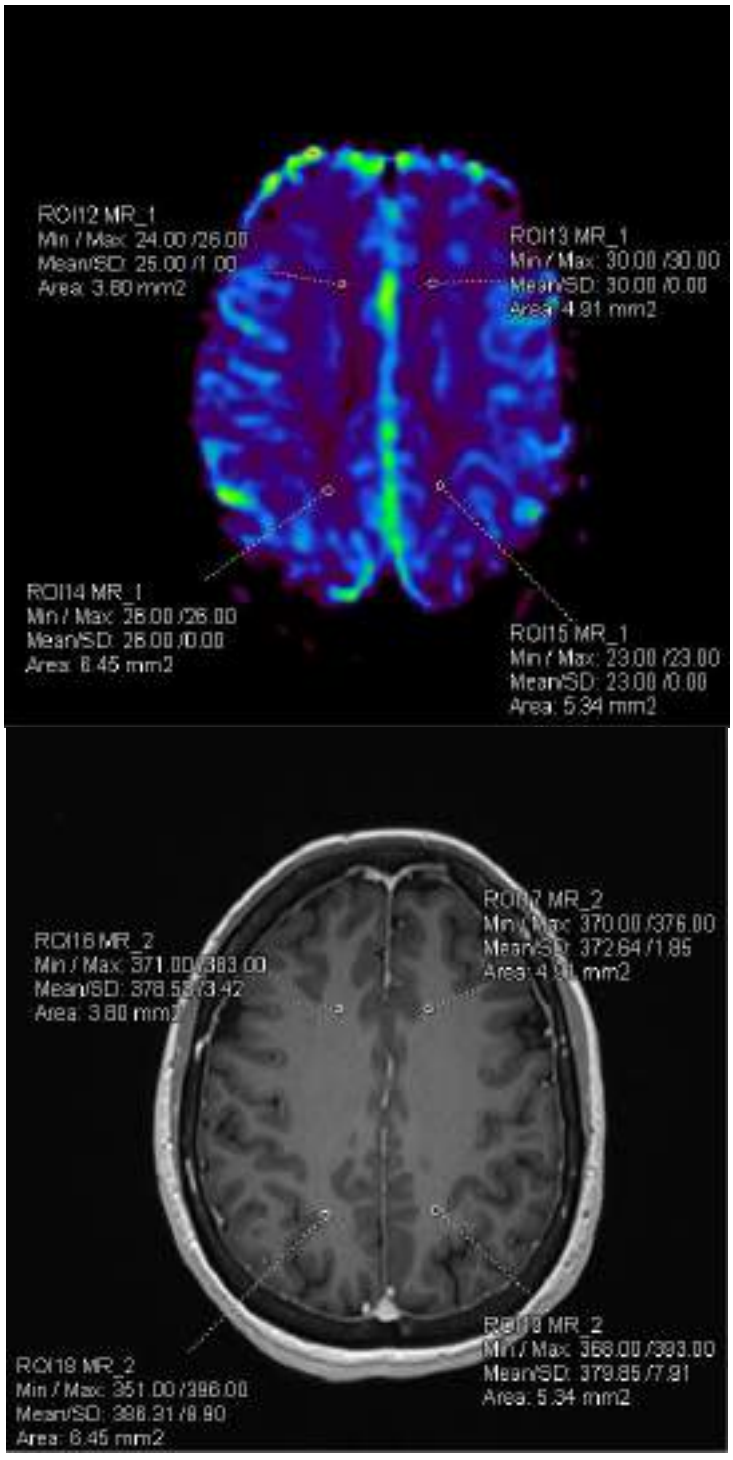

Figure 26b: Case 2 with normalised CBF for right and left frontal region is 0.37 and 0.45 respectively

Normalised cbf for right and left parietal region is 0.39 and 0.34 respectively

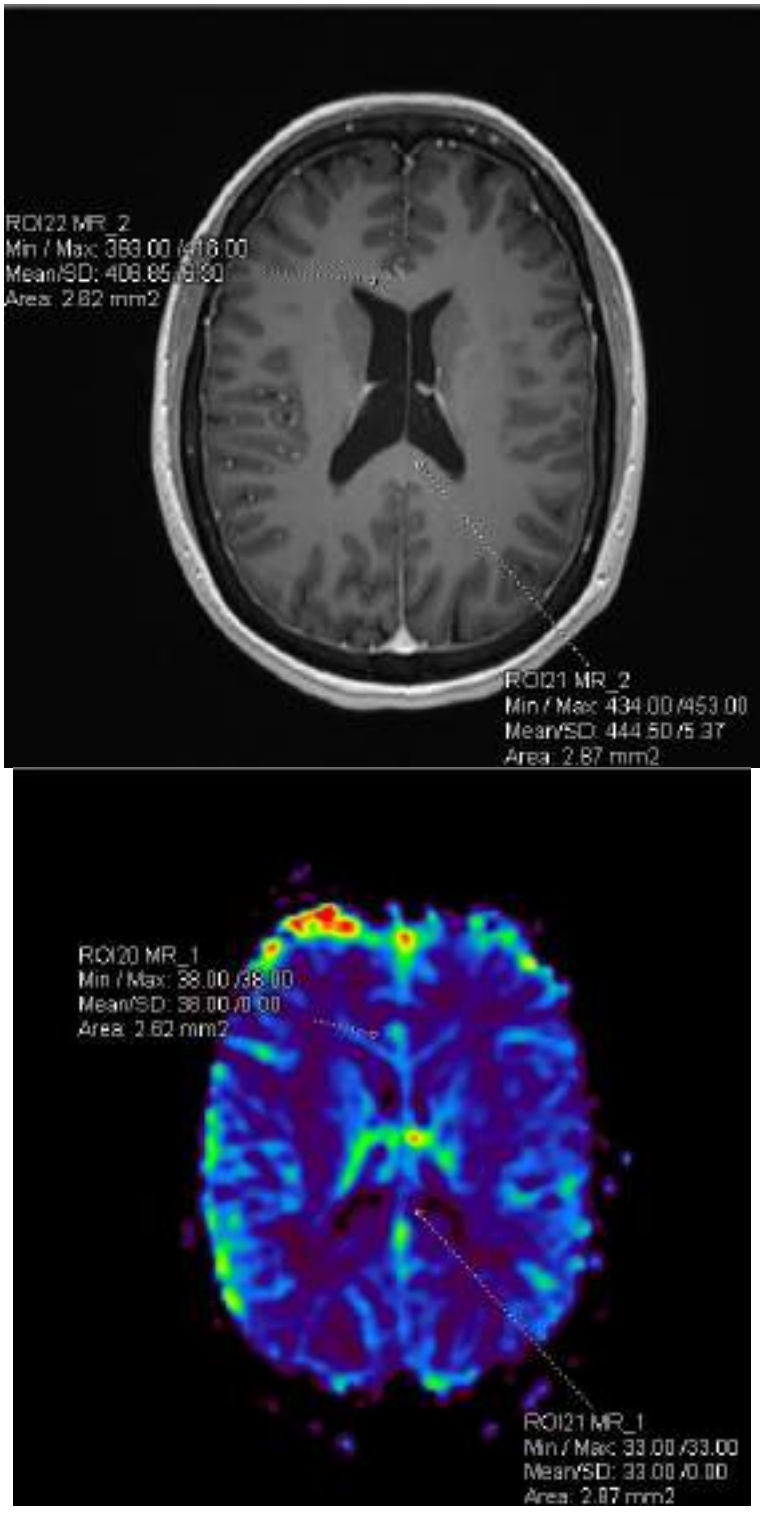

Figure 26c: Case 2 with normalised CBF of genu and splenium and respectively 0.5 and 0.50 respectively

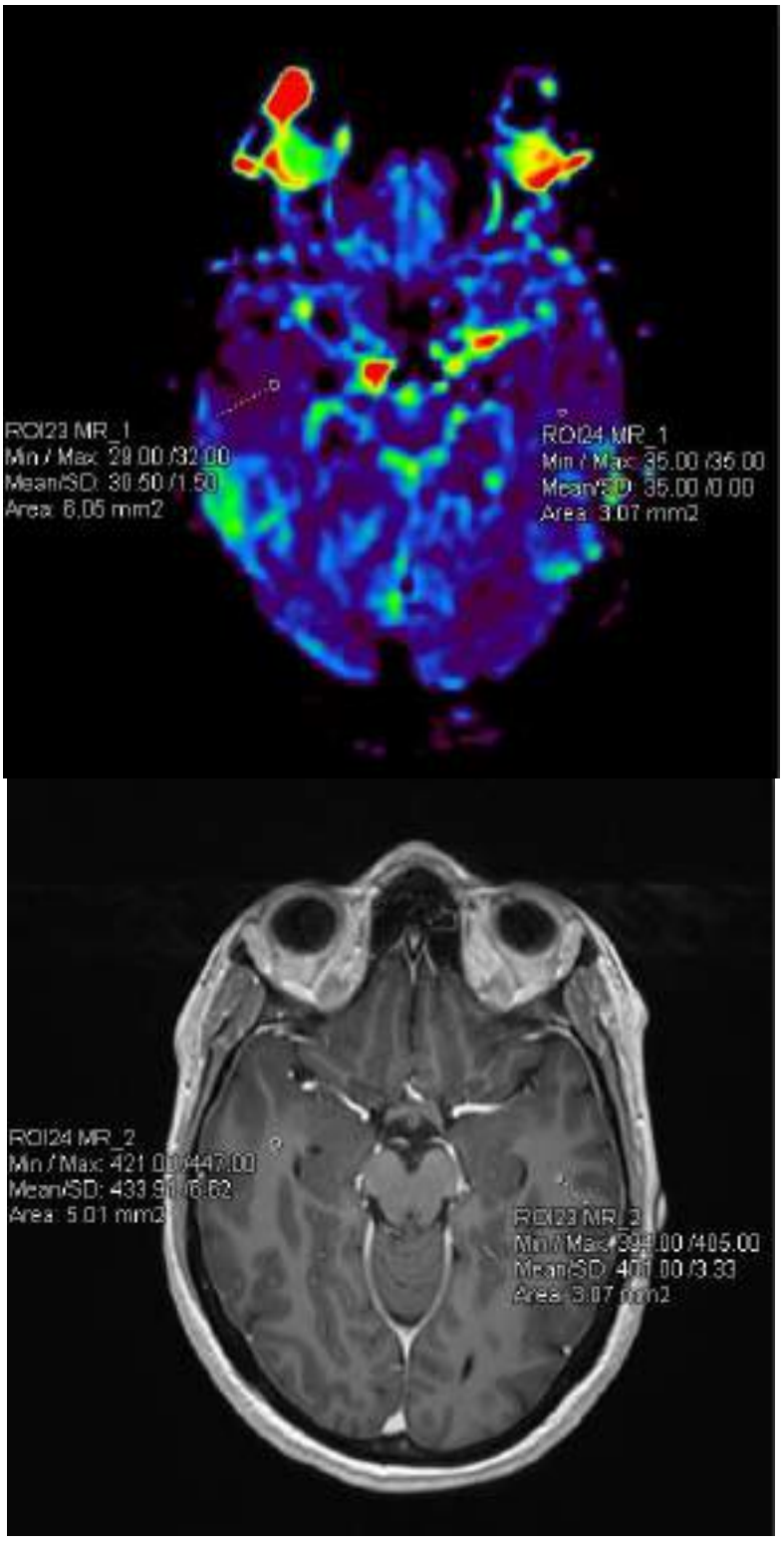

Figure 26d: Case 2 with normalised CBF for right and left temporo-occipital regions is 0.45 and 0.53 respectively

DISCUSSION

Myelin plaques are easily apparent on conventional MRI sequences. However apart from focal MS there occurs diffuse white matter injury outside the plaque which may appear macroscopically normal. Also, there is modest correlation of MRI visible lesions with neurological deficit.

This could be due to the fact that the changes in normal appearing white matter go undetected. Therefore nonconventional imaging, which quantitatively detects alterations in NAWM could be helpful, in prognosticating the patient, monitoring disease response and modulating the treatment.

In our study we have quantified Myelin volume and Perfusion parameters (CBF and CBV) in NAWM using Synthetic MR (SyMRI) QRAPMASTER pulse sequences (quantification of relaxation times and proton density by multiecho acquisition of saturation-recovery using turbo spinecho readout) and DSC MRI respectively.

Recent study by Laetitia Saccenti (2017) et al. compared the newer technique of SyMRI with magnetization transfer saturation (MTsat), T1-weighted images divided by T2-weighted images (T1w/T2w), and radial diffusivity (RD) in patients with MS. Strong correlations were found between SyMRIMVF and T1w/T2w, MTsat or RD for all the segmented ROIs. In each technique, the average estimated myelin differed significantly among regions, but the percentage change of NAWM from both periplaque white matter and plaque were highest in SyMRIMVF. It was supported by evidence that SyMRIMVF might be suitable for myelin evaluation in MS patients, with relevant resuIts as compared to other well-studied techniques. Moreover, it presented better sensitivity for the detection of the difference between plaque or periplaque white matter and NAWM. Hence SyMRI is comparable to other techniques of myelin estimation.[49]

Our ROI-based analysis of 8 specific NAWM regions found a significant difference in Myelin correlated component(MyC) in all regions of interest. Myelin correlated component is the mean percentage of Myelin in that ROI . MyC of NAWM was 31.95 % as compared to controls which was 36.66%. The

maximum difference was seen in frontal NAWM, while the least difference was found with genu and splenium of corpus callosum, the most myelinated white matter of brain. The mean MyC was 14.8% lower than the controls

The results are in concordance with previous study done by A.Hagiwara (2017) using tissue relaxometry. Hagiwara concluded the MVF(Myelin volume fraction) of NAWM in patients of MS was 30.96±3.74%. The average myelin volume of plaque was 5.97±4.86%. He further concluded that the difference in Myelin volume in plaques and periplaque white matter as compared to NAWM was 80.78±15.27% and 16.19 ±14.82% respectively. No significant correlation was found between MVF , EDSS and disease duration. [21]

Our results have been supported by previous histopathological correlations suggesting demyelination in NAWM of MS patients (Allen IV 1981) . Allen et al did a post mortem histopathological analysis of 14 MS cases and 11 controls. The results revealed significant gliosis, demyelination, small round cell infiltration, macrophage deposition and perivascular cuffing in MS patients as compared to controls.[6]

Previous study by Wallace W. tourtellotte and Julius Parker has (1968) suggested that since myelin is rich in lipid, it constitutes most of the lipid found in white matter. Therefore total lipid determination could be used as a simple indicator for myelin . It was found that NAWM in MS patients showed a 15.5% reduction in total lipid when compared with control white matter, suggesting a similar decrease in NAWM myelin. [50]

These results also correlate with other non conventional MRI techniques, like Myelin water imaging(MWI) to evaluate Myelin water fraction(MWF) using multi echo- spin echo(MESE) T2 relaxometry technique by Kitzler(2011)[36] and Tobias djamsched(2016) . Tobias et al found that MWF was significantly reduced in majority of NAWM ROI locations except for genu and splenium of corpus callosum. The mean MWF was 37 % lower as compared to controls. No correlation was found for disease duration or EDSS.[38]

Laule et al. (2004) described a 16% reduction of the MWF in the NAWM of a very heterogeneous study cohort. Laule et al concluded that reduced myelin

water fraction is attributed to reduced myelin and increased water content; latter being either due to inflammation or edema. However demyelination is a predominant pathology in NAWM. [35]

Kolind et al (2012) reported a 6% reduction of the MWF in the NAWM of PPMS-patients related to HCs using mcDESPOT. [37]

Choi et al(2018) quantified MWF using newer technique of myelin water imaging, i.e GRASE and ViSTa, with reduced MWF in all regions and correlation of MWF with disease duration. [41]

The disease duration was significantly correlated with Mean MyC (Pearson's correlation coefficient r= -0.48, p= 0.03).The results support the possibility of progressive demyelination in the NAWM over a long period in MS. This could also suggest a slowly progressing underlying pathological mechanism which is contributing to disease progression. This has been supported by Irene M Vavasour((2018) et al who did a longitudinal study on a cohort of 11 patients of MS and 4 controls. The mean MWF was 8 % lower over a duration of 5 years compared to baseline. This indicates that chronic, progressive myelin damage is an evolving process occurring over many years . It could also be attributed to wallerian degeneration along axons damaged by lesions, a phenomenon that is well documented in MS white matter.[40]

Mean MyC is significantly correlated with number of lesions, showing negative correlation with Pearson correlation coefficient of 0.68 and r^2=0.46.

No significant correlation was found between EDSS and Myelin volume in our study. This can be explained by the fact that we had a smaller cohort of patients with narrow range of EDSS(1-6.5) . 60 % of our patients had EDSS less than 2. Also, EDSS measurements are mainly weighted towards over locomotor disability, which is likely to depend on spinal cord damage(Kurtzke, 1983) . So larger groups of patients with a wide range of EDSS must be warranted to establish a correlation between myelin loss and EDSS. Laule (2004) also found no significant correlation of Myelin water fraction with EDSS[35]

A study by Mina Park et al (2019) correlates loss of myelin with cognitive decline in patients using quantitative synthetic MRI. The myelin partial volume in patients with cognitive decline was lower in those with white matter hyperintensities(37.5± 2.7%) than in the WMHs (-) group (37.5± 2.7%). Hence further studies can help us make a correlation between cognitive decline and myelin degradation in patients with MS.[51]

Our study revealed reduced perfusion parameters i.e. CBF and CBV in patients with MS in NAWM in all the regions of interest. Mean normalized CBV of NAWM was 0.40±0.07 as compared to controls which was 0.59±0.07. The mean normalized CBF of NAWM was 0.40±0.07 as compared to controls which was 0.55±0.07.

The changes in CBF and CBV have been supported by previous studies by Meng Law et al.(2004) who concluded that each region of NAWM had significantly reduced CBF and prolonged MTT compared to controls. These findings of diminished perfusion in NAWM is a evidence that MS has a primary vascular pathogenesis. No change in CBV was however found. This was explained by the compensatory vasodilatation in response to reduced CBF and CBV not low enough to cause ischemia or infarction. [46]

Adams et al(1985) proves our hypothesis histopathologically in his study where he explains the lymphocytic infiltration of veins; suggesting that MS is a subacute or chronic form of vasculitis that could precede lesion development.[33]

Arnold et al (1984) found lymphocytic retinal periphlebitis in absence of parenchymal findings, which indicate that vascular changes can occur independent of demyelination. [52]

Putnam(1937) also had similar results where he said that vascular insult occurs as a precursor to demyelination. [53]

Sumita Adhya et al(2006) determined regional patterns of perfusion abnormalities in NAWM in MS patients. The CBF and CBV were significantly lower than that of controls. These findings suggest that neuroinflammation will result in microvascular damage by differerent mechanism.

Lassmann (2003) said that inflammation leads onto edema, which can disturb the microcirculation. [32]

Nuclear studies by brooks et al (1984) have revealed that there are areas of reduced perfusion in both grey and white matter in patients of MS. [54]

Rashid et al (2004) used ASL (Arterial spin labeling) technique and stated that there were areas of reduced perfusion in cortical and deep grey matter structures consistent with axonal loss. Also, white matter showed increased perfusion values in patients not on immunomodulation therapy, which could be either due to increased cell activity or metabolic demand. It was the only study which demonstrated increased WM perfusion in NAWM. [43]

Jens wuerfel(2004) et al demonstrated foci of increased perfusion seen 3 weeks before the breakdown of blood brain barrier and the appearance of new lesion, which indicates that altered vascular dynamics will precede parenchymal inflammation. [55]

No significant correlation was found between EDSS and disease duration. The absence of correlation can be due to lack of sensitivity of these outcome measures to predict disability as reported by Hyland M et al but he also stated that the significance of perfusion alteration is not in predicting deficit, but in detecting acute inflammatory changes in MS that could detect new lesions or symptomatic lesions which will impact disability. [56]

Many studies have failed to establish a relationship between perfusion alteration and clinical outcomes of disability (Inglese et al[57], Debernard et al[58], Rashid et al[43].

A linear correlation was found between Normalized CBF and Normalized CBV(Pearson correlation of correlation coefficient of 0.514, r^2= 0.25) This has been supported by previous study by Sette et al. (1989)who performed a PET scan and found a significant linear correlation between CBF and CBV.[59]

Singer et al also performed perfusion MRI and indicated that relative CBF changed proportionately with relative CBV. [60]

Given that demyelination is a primary pathophysiological process in multiple sclerosis, monitoring the extent of myelin loss early in disease and quantifying

it will benefit people living with Multiple sclerosis. It would enable us to initiate and titrate the treatment early; monitor the response to treatment and personalize treatment according to the degree of myelin loss. When neither the lesion volume nor the lesion load correlates with the deficit or response to treatment in patients with MS, quantifying myelin could serve as a potential marker. A previous study by EM King et al (2018) [61] calculated MWF ratio i.e. the ratio of MWF of lesion to MWF of NAWM. This ratio was significantly correlated with the downslope walking intervention in MS patients having less myelin degradation. They said that this ratio might serve as potential neural biomarker to identify responders to intervention targeting functional impairments in MS.

However, future work is required to determine if a threshold MWF ratio for intervention response exists. Further studies are warranted to determine how generalizable the predictive value of myelin is to other rehabilitative or pharmacological interventions.

But to conclude myelin status could serve as a potential marker to initiate treatment early in course of disease and to monitor response, thus further prognosis of disease.

The main limiting factors in our study was a smaller cohort. A larger group of patients are required to validate the study in future. Our study focused on T2w WM hyperintense lesions without taking the T1w signal and contrast-enhancement (which requires the administration of a gadolinium-based contrast agent) into account when examining the activity of lesions. In the future, contrast-enhanced images could be used to separate subgroups or inflammatory lesions and study the differences in the metrics. Shorter duration of study also served as another limiting factor. A longitudinal study where we could analyze and predict the inflammatory perfusion alterations prior to formation of new lesions will allow us to initiate treatment early. We can also assess the change in myelin and perfusion over a gradual course of time. Our study did not take into consideration the fact that elderly patients have small vessel disease which could alter quantification of perfusion as well as myelin

values. Manual delineation of lesions was another limiting factor and the fact that we did not take into account the lesion load in spinal cord.

SUMMARY AND CONCLUSION

1. The mean MyC for all 8 ROI's in NAWM in MS patients was 31.95±1.67% as compared to controls which was 36.66±1.47%
2. The result is significant for all 8 ROI's with $p< 0.05$
3. The mean MyC is 14.8% lower than that of controls
4. Mean MyC is significantly correlated with disease duration, showing a negative correlation. The Pearson correlation coefficient is 0.48 with $r^2 =0.23$
5. Mean MyC is significantly correlated with number of lesions, showing negative correlation. The Pearson correlation coefficient is 0.68 with $r^2=0.46$
6. No significant correlation was found between Mean MyC and EDSS
7. The mean normalized CBV of NAWM in MS patients is 0.40±0.07 as compared to controls which is 0.59±0.07
8. The mean normalized CBF of NAWM was 0.40±0.07 as compared to controls which was 0.55±0.07.
9. The value of perfusion parameters is significantly lower in all regions of interest($p<0.05$)
10. No significant correlation was found between perfusion parameters, EDSS, disease duration and lesion load
11. nCBF was positively correlated with nCBV with Pearson correlation coefficient of 0.514 and $r^2=0.26$

CONCLUSION

To conclude, microstructural abnormalities occur in NAWM even before they are visible on routine MR sequences. Loss of myelin and reduced perfusion parameters precede plaque formation and structural atrophy in MS patients. Quantitatively assessing myelin loss could serve as a potential biomarker to determine the course of disease. It could help clinicians initiate and titrate the treatment in MS patients early and to personalize treatment and monitor response in each patient. A better understanding of the relationship between perfusion alterations, MS and clinical outcomes may be important to provide

new potential biomarkers for the assessment of pharmacological and rehabilitation intervention effects. Indeed, besides an epiphenomenon due to atrophy, hypoperfusion may be involved in MS pathogenetic mechanisms.

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KEY TO MASTER CHART

EDSS	Expanded disability status scale
MyC	Myelin correlated component
nCBF	Normalised cerebral blood flow
nCBV	Normalised cerebral blood volume

Sr.No	NAME	MRD	Group		AGE	SEX	DISEASE DURATION	STAGE	EDSS	Annualised relapse rate	LESIONS	LESIONS	MyC										
													Mean MyC	R. FRONTAL	L. FRONTAL	R. PARIETAL	L. PARIETAL	R. TEMPORO-OCCIPITAL	L. TEMPORO-OCCIPITAL	GENU	SPLENIUM	Plaque	R. FRONTAL
1	V	339831	Cases	1	47	M	8.00	RRMS	1	nil	40-50	50	29.4375	24.7	26.7	22.7	21.4	32.3	31.8	38.2	37.7	2.6	0.5
2	S	521992	Cases	1	22	M	4.00	RRMS	1	1	20-30	30	32.7	30.9	29.7	28.3	31.1	32.6	32.7	37.2	39.1	5.9	0.37
3	J	139604	Cases	1	37	M	6.00	RRMS	2	Nil	0-10	10	33.8	29.5	29.5	31.9	30.7	33.8	34.3	41.9	38.8	0.7	0.32
4	M	660436	Cases	1	36	F	4.00	RRMS	5.5	1	80-90	90	30.4375	28.9	28.4	28.6	28.1	29.7	30.1	35.6	34.1	1.2	0.42
5	G	1007954	Cases	1	22	M	0.33	RRMS	6	3	60-70	70	30.875	28.3	27.4	29	30.3	33.6	32.8	35.6	30	6.3	0.45
6	G	921034	Cases	1	30	M	10.00	RRMS	6	0.2	0-10	10	31.0375	29.1	28.7	30.6	27.5	30.5	31	35.8	35.1	0.6	0.36
7	G	996826	Cases	1	37	F	1.00	RRMS	2	2	0-10	10	34.825	35	34	33	32.6	35.6	35.5	34.6	38.3	0.5	0.2
8	S	952325	Cases	1	50	F	2.00	RRMS	2	0.5	0-10	10	33.5125	32.9	31.8	30.6	33.1	31.6	29.7	40.5	37.9	1.7	0.36
9	M	1019783	Cases	1	33	F	0.50	RRMS	3	2	30-40	40	31.675	30.7	31.5	28.1	27.5	31.7	29.7	39	35.2	2.1	0.3
10	K	966864	Cases	1	35	F	0.33	RRMS	1	2	10 to 20	20	34.5375	35.1	31.4	33.4	33.9	34.8	29.7	44.2	33.8	1.3	0.37
11	B	811559	Cases	1	25	F	0.50	RRMS	3	8	10 to 20	20	32.2875	29.3	30.5	31.5	32.1	31.2	29.7	40.2	33.8	1.3	0.41
12	S	979158	Cases	1	29	F	4.00	RRMS	2	0.5	40-50	50	32.625	29.1	29.3	34.6	33.3	32.1	29.7	39.1	33.8	0.4	0.53
13	CP	58936	Cases	1	14	F	10.00	RRMS	2	0.2	20-30	30	31.7	30.2	30.9	29.3	30.2	31.2	29.7	38.3	33.8	0.5	0.35
14	P	990280	Cases	1	16	F	0.25	RRMS	1	nil	10 to 20	20	32.1875	31.1	30.7	30.1	31.6	33.5	29.7	37	33.8	1.3	0.34
15	S	915878	Cases	1	35	F	10.00	SPMS	6.5	0.6	30-40	40	31.275	30.5	30.3	26.4	27.5	33.5	29.7	38.5	33.8	0.8	0.37
16	A	477518	Cases	1	39	M	10.00	RRMS	2	0.5	30-40	40	29.1375	28.2	23.6	25.6	27.6	27.7	29.7	36.9	33.8	0.6	0.4
17	K	963250	Cases	1	32	M	10.00	RRMS	2	nil	80-90	90	29	24.1	26.7	27.1	28.2	27.8	29.7	34.6	33.8	0.6	0.44
18	SK	1027214	Cases	1	22	M	3.00	RRMS	2	1	20-30	30	32.45	30.8	30.7	31.8	30.1	32.6	29.7	40.1	33.8	0.9	0.56
19	MJ	1144254	Cases	1	40	F	10.00	RRMS	3	0.1	0-10	10	33.5875	33.4	32	33.8	30.2	36.6	29.7	39.2	33.8	0.9	0.25
20	P	552961	Cases	1	36	F	5.00	RRMS	3	1	0-10	10	31.825	30.8	29	31.3	30.9	32.7	29.7	36.4	33.8	4.2	0.36
21	A	635870	Control	2	51	M	-	-	-	-	-	-	37.875	38.2	39.9	33.5	34	38	36.7	42.5	40.2	-	0.6
22	R	995755	Control	2	48	F	-	-	-	-	-	-	35.4625	35.3	35	36	34.4	36.4	35.7	36.6	34.3	-	0.43
23	A	653309	Control	2	33	M	-	-	-	-	-	-	38.5125	37.3	38.4	38.4	36.7	39.2	36.1	42.7	39.3	-	0.7
24	D	800599	Control	2	22	F	-	-	-	-	-	-	37.25	39.6	40	36	34.9	36.3	36.8	38.1	36.3	-	0.63
25	P	1030010	Control	2	18	F	-	-	-	-	-	-	37.675	37.2	38.4	36	34.7	36.2	37.7	42.2	39	-	0.56
26	K	988692	Control	2	22	M	-	-	-	-	-	-	35.8625	36.2	38.7	33	33.1	36.2	35.9	38	35.8	-	0.59
27	CR	264275	Control	2	58	M	-	-	-	-	-	-	34.3875	31.5	30.7	34.2	32	32.2	32.4	43.1	39	-	0.56
28	OP	1007593	Control	2	47	M	-	-	-	-	-	-	36.825	36.1	36.7	34.1	33.7	34.4	34.7	43.7	41.2	-	0.46
29	AS	884398	Control	2	19	F	-	-	-	-	-	-	36	33.5	34	34.3	33.9	34.5	40	42.7	35.1	-	0.57
30	DD	1035336	Control	2	62	F	-	-	-	-	-	-	35.35	34.6	34.3	34.6	34.1	35.6	33.4	40.8	35.4	-	0.47
31	SS	1014370	Control	2	39	F	-	-	-	-	-	-	35.75	37	34.9	34.3	34.6	35.4	36	39.8	34	-	0.56
32	RS	1036499	Control	2	32	M	-	-	-	-	-	-	37.4625	39	37.3	35.2	36.3	39.4	38.8	36.5	37.2	-	0.6
33	JS	660805	Control	2	26	M	-	-	-	-	-	-	36.4625	36.1	34.6	35.9	35.6	36.5	35.9	39	38.1	-	0.57
34	KK	1034924	Control	2	62	F	-	-	-	-	-	-	34.5875	32.5	33	32.7	33.4	33.5	33	38.6	40	-	0.57
35	AK	1034470	Control	2	29	F	-	-	-	-	-	-	40.4	40.9	39.5	39.9	39.7	41.4	40.9	40.5	40.4	-	0.58
36	PK	615478	Control	2	55	F	-	-	-	-	-	-	36.2	35.2	34.5	34.9	34.2	38.3	37.2	38.2	37.1	-	0.69
37	PG	186441	Control	2	30	M	-	-	-	-	-	-	36.3	35.8	35.9	33.9	34.6	35	36.4	41.2	37.6	-	0.47
38	BS	1153690	Control	2	54	M	-	-	-	-	-	-	37.55	38.9	37.6	34	33.5	37.9	35.2	39.8	43.5	-	0.68

Sr.No	NAME	MRD	nCBV									nCBF									
			L. FRONTAL	R. PARIETAL	L. PARIETAL	R. TEMPORO-OCCIPITAL	L. TEMPORO-OCCIPITAL	GENU	BODY	SPLENIUM	Mean nCBV	R. FRONTAL	L. FRONTAL	R. PARIETAL	L. PARIETAL	R. TEMPORO-OCCIPITAL	L. TEMPORO-OCCIPITAL	GENU	BODY	SPLENIUM	Mean nCBF
1	V	339831	0.43	0.5	0.53	0.47	0.51	0.6	0.8	0.57	0.54	0.4	0.42	0.38	0.4	0.36	0.37	0.46	0.48	0.45	0.41
2	S	521992	0.28	0.39	0.34	0.32	0.36	0.28	0.35	0.29	0.33	0.3	0.4	0.3	0.3	0.3	0.3	0.5	0.5	0.6	0.38
3	J	139604	0.45	0.37	0.45	0.37	0.25	0.4	0.55	0.5	0.4	0.48	0.4	0.36	0.37	0.4	0.4	0.47	0.43	0.5	0.42
4	M	660436	0.4	0.47	0.41	0.43	0.44	0.44	0.44	0.48	0.42	0.37	0.44	0.36	0.29	0.35	0.38	0.58	0.41	0.45	0.4
5	G	1007954	0.54	0.4	0.37	0.5	0.45	0.65	0.4	0.52	0.47	0.38	0.36	0.41	0.32	0.26	0.34	0.38	0.36	0.52	0.37
6	G	921034	0.4	0.35	0.4	0.38	0.4	0.45	0.5	0.37	0.4	0.3	0.35	0.32	0.38	0.37	0.26	0.24	0.4	0.23	0.31
7	G	996826	0.25	0.25	0.18	0.2	0.25	0.12	0.25	0.17	0.2	0.25	0.3	0.3	0.2	0.35	0.5	0.25	0.2	0.2	0.28
8	S	952325	0.36	0.32	0.37	0.31	0.29	0.35	0.36	0.38	0.34	0.34	0.4	0.29	0.28	0.33	0.36	0.32	0.35	0.36	0.33
9	M	1019783	0.42	0.34	0.3	0.24	0.29	0.35	0.37	0.32	0.32	0.4	0.45	0.37	0.4	0.5	0.5	0.38	0.36	0.38	0.47
10	K	966864	0.25	0.3	0.29	0.32	0.33	0.5	0.47	0.59	0.37	0.34	0.4	0.36	0.38	0.36	0.38	0.4	0.34	0.42	0.37
11	B	811559	0.41	0.31	0.2	0.35	0.2	0.45	0.41	0.6	0.37	0.4	0.4	0.32	0.3	0.32	0.4	0.5	0.52	0.48	0.4
12	S	979158	0.4	0.42	0.3	0.46	0.48	0.55	0.5	0.43	0.4	0.42	0.44	0.46	0.4	0.4	0.42	0.57	0.42	0.47	0.44
13	CP	58936	0.36	0.38	0.4	0.3	0.37	0.5	0.47	0.56	0.41	0.35	0.25	0.35	0.29	0.26	0.32	0.51	0.45	0.5	0.36
14	P	990280	0.33	0.37	0.34	0.39	0.32	0.49	0.4	0.65	0.44	0.38	0.4	0.43	0.47	0.43	0.38	0.6	0.6	0.5	0.46
15	S	915878	0.4	0.35	0.36	0.36	0.36	0.56	0.68	0.52	0.44	0.3	0.38	0.34	0.36	0.33	0.42	0.57	0.6	0.6	0.44
16	A	477518	0.38	0.25	0.43	0.42	0.37	0.48	0.49	0.44	0.4	0.45	0.56	0.42	0.4	0.42	0.51	0.46	0.6	0.52	0.48
17	K	963250	0.36	0.36	0.3	0.38	0.42	0.56	0.5	0.42	0.41	0.46	0.44	0.4	0.42	0.38	0.42	0.48	0.52	0.38	0.43
18	SK	1027214	0.58	0.51	0.5	0.4	0.46	0.62	0.62	0.58	0.53	0.48	0.5	0.55	0.44	0.5	0.47	0.55	0.44	0.6	0.5
19	MJ	1144254	0.3	0.25	0.32	0.25	0.28	0.53	0.57	0.62	0.37	0.3	0.35	0.35	0.47	0.3	0.39	0.55	0.53	0.57	0.42
20	P	552961	0.32	0.42	0.38	0.29	0.34	0.46	0.5	0.48	0.39	0.33	0.35	0.39	0.42	0.38	0.35	0.46	0.5	0.46	0.4
21	A	635870	0.7	0.75	0.72	0.48	0.6	0.72	0.61	0.87	0.67	0.53	0.59	0.61	0.73	0.69	0.53	0.53	0.5	0.66	0.6
22	R	995755	0.58	0.43	0.54	0.5	0.52	0.48	0.5	0.58	0.5	0.4	0.52	0.54	0.48	0.46	0.5	0.52	0.56	0.8	0.53
23	A	653309	0.7	0.66	0.8	0.6	0.7	0.8	0.7	0.6	0.7	0.6	0.61	0.76	0.69	0.6	0.64	0.8	0.69	0.61	0.6
24	D	800599	0.75	0.5	0.6	0.55	0.58	0.58	0.8	0.76	0.63	0.52	0.46	0.58	0.64	0.62	0.58	0.6	0.6	0.6	0.57
25	P	1030010	0.61	0.52	0.54	0.56	0.54	0.5	0.6	0.52	0.55	0.66	0.68	0.57	0.64	0.64	0.62	0.6	0.68	0.61	0.63
26	K	988692	0.42	0.53	0.6	0.5	0.42	0.4	0.42	0.47	0.49	0.4	0.48	0.4	0.4	0.47	0.5	0.53	0.58	0.64	0.49
27	CR	264275	0.52	0.48	0.62	0.64	0.7	0.72	0.7	0.56	0.61	0.51	0.59	0.57	0.55	0.7	0.7	0.68	0.63	0.72	0.62
28	OP	1007593	0.53	0.44	0.51	0.55	0.55	0.44	0.46	0.48	0.49	0.52	0.54	0.55	0.52	0.5	0.54	0.5	0.54	0.51	0.51
29	AS	884398	0.71	0.62	0.71	0.68	0.66	0.62	0.64	0.66	0.66	0.56	0.53	0.55	0.55	0.56	0.59	0.58	0.64	0.68	0.58
30	DD	1035336	0.52	0.47	0.55	0.55	0.61	0.63	0.61	0.6	0.55	0.45	0.47	0.51	0.5	0.5	0.46	0.5	0.51	0.5	0.48
31	SS	1014370	0.6	0.6	0.64	0.6	0.6	0.68	0.64	0.5	0.6	0.63	0.53	0.61	0.53	0.53	0.67	0.72	0.67	0.69	0.62
32	RS	1036499	0.63	0.58	0.6	0.6	0.68	0.65	0.68	0.68	0.63	0.53	0.5	0.5	0.54	0.5	0.46	0.5	0.48	0.48	0.5
33	JS	660805	0.54	0.54	0.53	0.51	0.53	0.58	0.56	0.6	0.55	0.53	0.55	0.52	0.46	0.46	0.49	0.53	0.53	0.49	0.5
34	KK	1034924	0.6	0.62	0.58	0.65	0.6	0.6	0.65	0.58	0.6	0.6	0.7	0.5	0.6	0.6	0.5	0.6	0.42	0.54	0.57
35	AK	1034470	0.54	0.56	0.53	0.54	0.6	0.58	0.65	0.62	0.57	0.58	0.56	0.56	0.58	0.52	0.59	0.6	0.58	0.54	0.57
36	PK	615478	0.63	0.6	0.63	0.58	0.6	0.72	0.6	0.8	0.64	0.46	0.48	0.5	0.49	0.46	0.48	0.5	0.52	0.5	0.49
37	PG	186441	0.43	0.54	0.4	0.49	0.4	0.36	0.54	0.45	0.45	0.41	0.4	0.41	0.39	0.41	0.48	0.37	0.37	0.39	0.4
38	BS	1153690	0.67	0.64	0.7	0.6	0.64	0.78	0.71	0.71	0.68	0.57	0.63	0.79	0.72	0.72	0.6	0.7	0.82	0.6	0.68

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Date 20/4/2021

The Principal,
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Subject: **Approval of Plan of Thesis.**

This is to inform you that the Faculty of Medical Sciences in its meeting held on 22.01.2021 has approved the plan of thesis of **Dr. Isha Sharma**, a student of your college for **MD Radio-Diagnosis** along with the names of Supervisor/Co-Supervisor as under:-

Topic Plan of Thesis
Myelin water and perfusion imaging in normal appearing white matter in patients with multiple sclerosis

Supervisor
Dr. Kavita Saggar
Prof. & Head,
Dept. of Radio-diagnosis,
DMCH, Ludhiana.

Co-Supervisor
Dr. Gagandeep Singh
Prof. & Head,
Dept. of Neurology,
DMCH, Ludhiana.

Dr. Ritu Dhawan Galhotra
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Dept. of Radio-diagnosis,

The student has to submit the three copies of thesis (along with PDF format) , to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She must directed to attach a photocopy of this letter with the forwarding letter while submitting thesis to the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic.(if any).

S P Singh

Dr. S P Singh MS(Oph)
Controller of Examinations

Note :

1. ***If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.***
2. *The student may submit the thesis as per the schedule already conveyed i.e for May/June examination 30th Nov of the preceding the year of examination and for Nov/Dec examinations 31st May of the year of examination.*

MYELIN WATER AND PERFUSION IMAGING IN NORMAL APPEARING WHITE MATTER IN PATIENTS WITH MULTIPLE SCLEROSIS

THESIS PROPOSAL

FOR APPROVAL OF SUBJECT OF THESIS TO BE SUBMITTED IN

PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MD

In

RADIODIAGNOSIS

OF THE

BABA FARID UNIVERSITY OF HEALTH SCIENCES

FARIDKOT

November, 2019

Dr. Isha Sharma

DEPARTMENT OF RADIODIAGNOSIS
DAYANAND MEDICAL COLLEGE AND HOSPITAL
LUDHIANA

ABSTRACT OF PLAN OF THESIS

Title	Myelin water and perfusion imaging in normal appearing white matter in patients with multiple sclerosis
For the degree of	MD (Radiodiagnosis)
Name of the candidate	Dr. Isha Sharma
Supervisor	Dr. Kavita Saggar, Professor & Head, Department of Radiodiagnosis, Dayanand Medical College & Hospital
Co-supervisor(s)	Dr. Gagandeep Singh, Professor & Head, Department of Neurology, Dayanand Medical College & Hospital Dr. Ritu Dhawan Galhotra, Professor, Department of Radiodiagnosis, Dayanand Medical College & Hospital
Institution	Dayanand Medical College and Hospital, Ludhiana
University	Baba Farid University of Health Sciences, Faridkot

INTRODUCTION: Multiple sclerosis is a chronic demyelinating disease of central nervous system (CNS) in which white matter (WM) lesions are visualized on T2 weighted (W) MRI in most patients. Normal appearing white matter (NAWM) are areas of brain and spinal cord which appear 'normal' on standard clinical imaging however show demyelination on histologic studies. In this study we would evaluate Myelin water fraction and perfusion parameters to evaluate early demyelination in NAWM of patients with multiple sclerosis.

AIM OF STUDY: 1) Evaluation of myelin water fraction and perfusion parameters in NAWM in patients of multiple sclerosis.

METHOD OF COLLECTION OF DATA: A prospective study will be done on patients with multiple sclerosis based on the revised McDonalds criteria 2017,referred to MRI department of Radiodiagnosis Dayanand medical college, Ludhiana and compared with healthy controls over a period of 1 and half years

INCLUSION CRITERIA: All patients with multiple sclerosis based on revised McDonalds criteria 2017

EXCLUSION CRITERIA: Patients with known contrast allergy or eGFR<30ml/min/1.73m2.

Any contraindications to MRI(patients with cardiac pacemakers, claustrophobic patients) and anyone unwilling to participate in the study.

CLINICAL SIGNIFICANCE: Myelin water fraction is significantly decreased in NAWM in MS, thus acts as surrogate marker to quantify invisible burden of disease and clinical disability. It can also distinguish between different subtypes of MS. Clinically, perfusion MR imaging could allow earlier detection of the acute focal inflammatory changes underlying relapses and new lesions, and could constitute a marker for cognitive dysfunction in MS.

Keywords: NAWM, MWF,Multiple sclerosis, perfusion

(Signature) Candidate | (Signature) Co-supervisor | (Signature) Co-supervisor | (Signature) Supervisor | (Signature) Principal

CERTIFICATE OF FACILITIES AVAILABLE

This is to certify that the facilities for work on the subject of thesis titled **"MYELIN WATER AND PERFUSION IMAGING IN NORMAL APPEARING WHITE MATTER IN PATIENTS WITH MULTIPLE SCLEROSIS"** exist in the Department of Radiodiagnosis, DMC&H, Ludhiana and will be provided to the candidate. We will see that the data being included in the thesis is genuine and is collected by the candidate herself under our supervision and guidance.

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6. BRIEF RESUME OF THE INTENDED WORK

6.1 NEED FOR THE STUDY

Multiple sclerosis is the most common neuroimmunological disorder. Onset is usually seen in young adults and the disease course is characterised by long survival and increasing disability over time.[1]

It is is a chronic demyelinating disease of the central nervous system in which breakdown of myelin is thought to be due to immune mediated inflammation.[2] Histopathologic and biochemical studies have revealed that areas of grossly normal-appearing white matter (NAWM) distant from lesions can exhibit loss of myelin-specific proteins , alterations in neurofilament phosphorylation, and infiltration by macrophages and T lymphocytes [3,4] These changes have been detected by advanced MRI approaches including T1 or T2 measurements, diffusion tensor imaging (DTI),magnetization transfer imaging (MTI), and myelin water imaging (MWI).[5]

Conventional T1- and T2weighted images have been used to identify MS lesions that often appear as T1 hypointensity and T2 hyperintensity signals in white matter (WM). Despite obvious contrasts between MS lesions and normal-appearing brain tissue on conventional images, direct interpretation of magnetic resonance (MR) images such as the lesion count or volume has not shown a strong correlation with clinical disability, suggesting that there exist other areas of the brain, such as normal-appearing white matter (NAWM) and gray matter, that may be affected by this disease.[5]

Interestingly, the abnormalities in the NAWM have been observed even before the formation of a visible lesion and have been shown to have a correlation with clinical disability.[6] It has also been used to understand the pathogenesis of MS. Therefore, a method that can reliably detect changes in the NAWM can be an important tool for exploring MS.

Myelin water imaging is an MR method that provides in vivo measurement of myelin. Histological validation work in both human brain and spinal cord tissue demonstrates a strong correlation between myelin water and staining for myelin, validating myelin water as a marker for myelin.[7]

The changes in MS NAWM are clinically relevant as they present very early in the course of the disease at the time of the first clinical presentation, and

they correlate with disability, cognitive impairment, and the degree of brain atrophy.[8]

Cerebral hypoperfusion and vascular factors are strictly involved in neurovascular dysfunction, vascular oxidative stress, and relative tissue hypoxia, well in advance of any demyelinating lesions.[9]

Very little is known about perfusion changes in the NAWM of patients with MS. Early studies with dynamic susceptibility contrast-enhanced (DSC) perfusion MRI (pMRI) focused on lesions and measured relative perfusion values expressed as a ratio to the perfusion of contralateral NAWM. However, NAWM is, in reality, abnormal in MS, and so makes an inappropriate reference. A more recent study employing absolute measures of flow and volume has reported decreased cerebral blood flow (CBF) in the NAWM of patients with relapsing–remitting (RR) MS. Conversely, elevation of CBF and cerebral blood volume (CBV) has been reported in NAWM of RR-MS patients several weeks before focal leakage of the blood–brain barrier (BBB) and plaque formation. Both these studies suggest that bolus tracking perfusion might provide a sensitive measurement of disease activity and treatment effect.[10]

An early recognition of the inflammatory process enables patients to begin treatment with an immunomodulatory agent even before the technical diagnosis of definite MS so that the degenerative progression of MS can be retarded.[11]

Therefore MWF can act as surrogate marker to quantify invisible burden of disease and clinical disability. It can also distinguish between different subtypes of MS. Clinically, perfusion MR imaging could allow earlier detection of the acute focal inflammatory changes underlying relapses and new lesions, and could constitute a marker for cognitive dysfunction in MS.

6.2 REVIEW OF LITERATURE

MYELIN WATER IMAGING

Kitzler(2012) took a 26 patient cohort There were 16 patients with definite MS: 5 relapsing–remitting (RRMS), 6 secondary-progressive (SPMS), and 5 primary-progressive (PPMS). In addition, 10 clinically isolated syndrome (CIS) patients were recruited. The mean myelin water fraction was compared in different white matter regions between MS patients and healthy controls .Mean MWF in both total WM ($p< 0.01$) and NAWM ($p<0.01$) was significantly lower than the corresponding mean WM MWF for healthy controls. Across all MS courses, the mean MWF value was significantly lower in DAWM and lesions than in WM of controls.[12]

Tobias Djamsched et al (2016) evaluated 17MS-patients and 14 age-matched healthy controls(HC)s using a 3T Magnetic Resonance Imaging (MRI) examination including MWI (8 slices, 12 minutes acquisition time), T2w and T1 pre and post gadolinium (GD) administration. Black holes (BH), contrast enhancing lesions (CEL) and T2 lesions were marked and registered to MWI. Additionally, regions of interest (ROI) were defined in the frontal, parietal and occipital normal appearing white matter (NAWM)/white matter (WM), the corticospinal tract (CST), the splenium (SCC) and genu (GCC) of the corpus callosum in patients and HCs. Mean values of myelin water fraction (MWF) were determined for each ROI. Significant differences ($p<0.05$) of the MWF were found in all three different MS-lesion types (BH, 134CEL, T2 lesions), compared to the WM of HCs. the NAWM of MS-patients compared to HCs was 37%.The mean MWF values among the different lesion types were significantly differing from each other. Comparing MS patients vs. HCs, we found a significant ($p<0.05$)difference of the MWF in all measured ROIs except of GCC and SCC.[13]

Shannon Kolind, Lucy Matthews et al(2016) imaged 17 PPMS patients and 17 age-matched controls using a whole-brain multi-component relaxation imaging technique to measure MWF. Analysis of MWF reduction was performed on three spatial levels: 1) histogram; 2) white matter skeleton; and 3) voxel-wise at the single-subject level. From histogram analysis, PPMS patients had significantly reduced global normal appearing white matter MWF

(6%, p= 0.04) compared to controls. Focal lesions showed lower MWF values than white matter in controls (61%, p<.001) and patients (59%, <0.001)[14]

PERFUSION STUDIES

W Rashid et al(2003) evaluated 60 patients (21 relapsing remitting, 14 secondary progressive, 12 primary progressive, 13 benign) and 34 healthy controls. Statistical parametric mapping (SPM '99) was used to investigate regional variations in perfusion in both GM and WM. Global WM perfusion was derived by segmenting WM from images using T1 relaxation times. Regions of lower perfusion in predominantly GM were observed in the primary and secondary progressive cohorts, particularly in the thalamus. Increased WM perfusion was seen in relapsing remitting and secondary progressive cohorts.[15]

Sumita Adhya, a Glyn Johnson et al(2006) aimed to determine regional pattern of tissue perfusion in the normal-appearing white matter (NAWM) of patients with primary-progressive (PP), relapsing-remitting (RR) multiple sclerosis (MS) and healthy controls, and to investigate the association between perfusion abnormalities and clinical disability. Using dynamic susceptibility contrast (DSC) perfusion MRI at 3 T, 22 patients with clinically definite MS, 11 with PP-MS and 11 with RR-MS and 11 age- and gender-matched healthy volunteers were included. Absolute cerebral blood flow (CBF), cerebral blood volume (CBV) and mean transit time (MTT) were measured in the periventricular, frontal, occipital NAWM and in the splenium of the corpus callosum. Compared to controls, CBF and CBV were significantly lower in all NAWM regions in both PP-MS patients and RR-MS patients and lower CBV in the periventricular and frontal NAWM (p values: 0.0029 and 0.022). EDSS was significantly correlated with the periventricular CBF (r<0.48, p= 0.0016) and with the periventricular and frontal CBV (r<0.42, p= 0.015; r<0.35, p= 0.038, respectively). This study suggests that the hemodynamic abnormalities of NAWM have clinical relevance in patients with MS.[10]

Miho Ota Et al(2013)evaluated, who 27 patients with MS and 24 healthy volunteers underwent magnetic resonance imaging and pCASL to assess CBF. Differences in CBF between the two groups and the relationships of CBF values with the T2-hyperintense volume were evaluated. Compared to

the healthy volunteers, reduced CBF was found in the bilateral thalami and right frontal region of the MS patients. The volume of the T2-hyperintense lesion was negatively correlated with regional CBF in some areas, such as both thalami. Results suggest that demyelinated lesions in MS mainly have a remote effect on the thalamus and that the measurement of CBF using ASL could be an objective marker for monitoring disease activity in MS.[11]

Maxim Bester et al (2015) evaluated 33 patients with relapsing-remitting MS using 3T MRI scans including dynamic susceptibility contrast perfusion-weighted MRI. Cerebral blood flow (CBF) and cerebral blood volume (CBV) were measured in normal appearing white matter. Patients were stratified in a high- and low-inflammatory group according to the number of new contrast enhancing lesions.13 were classified as high-inflammatory. Compared to low-inflammatory patients, the high-inflammatory group demonstrated significantly higher CBV ($p = 0.001$) and CBF ($p = 0.014$) values.[16]

Meng Law et al(2016) Dynamic susceptibility contrast-enhanced T2*-weighted MR imaging were performed in 17 patients with RRMS) and 17 control patients. Absolute cerebral blood volume (CBV), absolute cerebral blood flow (CBF), and mean transit time (MTT) (referenced to an arterial input function by using an automated method) were determined in periventricular, intermediate, and subcortical regions of NAWM at the level of the lateral ventricles. Each region of NAWM in patients with RR-MS had significantly decreased CBF ($P< .005$) and prolonged MTT ($P<.001$) compared with the corresponding region in control patients.[17]

Robert M. D'Ortenzio et al(2017)compared pseudo-continuous Arterial Spin Labeling CBF to the Bookend technique in a prospective cohort of 19 healthy controls, 19 relapsing-remitting MS subjects without cognitive impairment and 20 relapsing-remitting MS subjects with cognitive impairment on a voxel-wise and Brodmann regional basis. Voxel-wise paired T-tests revealed no significant CBF differences between techniques after normalization of global mean intensities. Highest Pearson correlations were observed in deep GM structures (average $r = 0.71$ basal ganglia, and $r = 0.65$ thalamus) but remained robust for cortical GM, WM and WML (average $r = 0.51, 0.53, 0.54$ respectively). Lower Pearson correlations were observed for CL (average $r=0.23$).[18]

Ping Yin et al (2018) evaluated 30 patients with multiple sclerosis (MS) who underwent conventional magnetic resonance imaging (MRI) and DCE-MRI with a 3.0 Tesla MR scanner. An extended Tofts linear model was used to quantitatively measure MR imaging biomarkers. The histogram parameters and correlation among imaging biomarkers, EDSS scores, and disease duration were also analyzed. It was observed that he MR imaging biomarkers volume transfer constant (K^{trans}), volume of the extravascular extracellular space per unit volume of tissue (Ve), fractional plasma volume (V_p), cerebral blood flow (CBF), and cerebral blood volume (CBV) of contrast-enhancing (CE) lesions were significantly higher ($P < 0.05$) than those of nonenhancing (NE) lesions and normal-appearing white matter (NAWM) regions.[19]

6.3 AIMS AND OBJECTIVES OF THE STUDY

1. Evaluation of Myelin water fraction and perfusion parameters in normal appearing white matter in patients of multiple sclerosis and its comparison with healthy controls.

7. MATERIALS AND METHODS

7.1 Source of Data

A prospective study will be done on patients diagnosed with Multiple sclerosis based on Revised McDonalds Criteria 2017 referred for MRI Brain to the department of Radiodiagnosis Dayanand medical college and hospital, Ludhiana during a 1 and a half year period. starting from 1 December 2019

7.2 METHOD OF COLLECTION OF DATA

Patients diagnosed with multiple sclerosis based on revised McDonalds criteria 2017 will be included in the study.10 Healthy controls (no imaging findings s/o white matter disease) will be included in the study. Normative data will be obtained from 10 healthy controls and compared with the data of patients of multiple sclerosis.

INCLUSION CRITERIA:

All patients diagnosed with multiple sclerosis based on revised McDonalds criteria 2017

EXCLUSION CRITERIA:

1)Any contraindications to MRI(patients with cardiac pacemakers claustrophobic patients.)

2)Anyone who is unwilling to participate in the study.

3))Anyone with known contrast allergy or eGFR<30ml/min/1.73m2

MRI IMAGING TECHNIQUE:

MR imaging will be performed on a 3T unit(Magnetom Skyra, Siemens, Germany.) using T1, T2, DWI, SWI, Contrast T1 WI, Dynamic susceptibility Contrast (DSC) imaging, Myelin Water Imaging(MWI)-Grase sequence

Sequence	T1 Axial	T2 Axial	T2 FLAIR Axial	T2 FLAIR Saggital	DWI	T1 Post Contrast	SWI	Grase	Perfusion Axial
Acquisition Time	1.22min	1.28min	2.08 min	3.42 min	2.03 min	5.38 min	1.28min	12.05 min	1.42min
Orientation	Transverse	Transvere	Transverse	Saggital	Transverse	Transverse	Transverse	Transverse	Transverse
Resolution Matrix	204x256	269x512	227x320	227x320	160x160	256x256	163x224	112x160	128x128
No. of slices	27	27	27	27	27	176/slab	60/slab	30/slab	20
FoV Read(mm)	220	220	220	220	220	230	220	240	220
FoV phase (%)	87.5	87.5	87.5	87.5	100	100	91.1	70	100
Slice thickness(mm)	4mm	4mm	4mm	4mm	4mm	1mm	2.5mm	5mm	4mm
Slice Gap(mm)	1.2mm	1.2mm	1.2mm	1.2mm	1.2mm	-	-	-	1.2mm
Repitition Time(msec)	2000msec	5060 msec	9000msec	9000msec	5500msec	2200msec	27msec	1000msec	1600msec
TE(msec)	8.6msec	91msec	81msec	81msec	64msec	2.48msec	20msec	10msec /1000msec	30msec
Flip Angle	150	120	130	130	180	8	15	90	90
Flow Compression	No	Read	No	No	-	No	Yes	-	

STATISTICAL ANALYSIS:

Data will be described in terms of range; mean ±standard deviation (±SD), median, frequencies (number of cases) and relative frequencies (percentages) as appropriate. Comparison of quantitative variables between the study groups will be done using Student t-test and Mann Whitney U test for independent samples for parametric and non-parametric data respectively. For comparing categorical data, Chi square ($\chi 2$) test will be performed and exact test was used when the expected frequency is less than 5. A probability value (p value) less than 0.05 will be considered statistically significant. All statistical calculations will be done using SPSS (Statistical Package for the Social Science) SPSS 21 version statistical program for Microsoft Windows.

OUTCOME:

- Myelin water fraction(MWF) and perfusion parameters will be calculated in normal appearing white matter of patients with multiple sclerosis controls to assess early demyelinating changes in the areas which appear otherwise normal on standard MR imaging.
- **7.3 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR INTERVENTIONS TO BE CONDUCTED ON PATIENTS OR OTHER HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEFLY.**

The study requires MRI brain to be done on patients with clinical and Radiological evidence of multiple sclerosis. However, the study does not require any intervention to be done on patients, other humans or animals.

7.4 HAS ETHICAL CLEARANCE BEEN OBTAINED FROM YOUR INSTITUTION IN CASE OF 7.3?

Attached herewith

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PROFORMA

S.no

Patient

Name:

Age:

Sex:

MRI regd. no. :

Address:

Phone no:

Stage of Multiple Sclerosis

Date of first clinical symptom

First clinical symptom

First clinical syndrome

No. of relapses

Annualised relapse rate

Presenting complaints:

1) Ambulation

2) Bowel/Bladder

3) Visual

4) Sensory

5) Pyramidal

Signature of Candidate

Signature of Supervisor

6) Ataxia

7) Mentation/Mood

8) Nystagmus/ Dysarthria

9) Other

Stage of disease progression:

EDSS score:

Date of diagnosis(based on the Revised McDonald's criteria 2017):

Disease duration:

Treatment:

CSF oligoclonal band assay (If available):

Previous MRI findings:

<u>Healthy control</u>

Patient:

Name:

Age:

Sex:

MRI regd. no. :

Address:

Signature of Candidate **Signature of Supervisor**

Phone no:

Presenting complaints:

MRI findings:

MRI FINDINGS IN PATIENTS WITH MULTIPLE SCLEROSIS AND HEALTHY CONTROLS

No.of white matter lesions in patients of multiple sclerosis-

MyelIn Water Imaging

Grase sequence	Patients		Healthy controls	
MWF in NAWM	R	L	R	L
Frontal WM				
Parietal WM				
Occipital WM				
Genu of corpus callosum				
Splenium of corpus callosum				
Body of corpus callosum				

Signature of Candidate

Signature of Supervisor

DSC sequence

	Patients		Healthy controls	
CBV in NAWM	R	L	R	L
Frontal WM				
Parietal WM				
Occipital WM				
Genu of corpus callosum				
Splenium of corpus callosum				
Body of corpus callosum				

DSC sequence

	Patients		Healthy controls	
CBF in NAWM	R	L	R	L
Frontal WM				
Parietal WM				
Occipital WM				
Genu of corpus callosum				
Splenium of corpus callosum				
Body of corpus callosum				

Signature of Candidate

Signature of Supervisor

DSC sequence

	Patients		Healthy controls	
MTT in NAWM	R	L	R	L
Frontal WM				
Parietal WM				
Occipital WM				
Genu of corpus callosum				
Splenium of corpus callosum				
Body of corpus callosum				

Signature of Candidate

Signature of Supervisor

INFORMED PATIENT CONSENT

Date: _____

I.....D/o, W/o, S/o..... .....am willing to participate in the study **"MYELIN WATER AND PERFUSION IMAGING IN NORMAL APPEARING WHITE MATTER IN PATIENTS WITH MULTIPLE SCLEROSIS"** The procedure with its complications has been explained to me in local vernacular language. I have fully understood that no special tests and extra expenditure in any form, merely for the purpose of thesis is being carried out. I am free to opt out the study any time without assigning any reason.

....

Signature of the Witness Signature of the patient

Name:

Signature of Candidate **Signature of Supervisor**

मरीज अनुमति फार्म

तिथि _____

मैं _____ पत्नी/पुत्र/पुत्री _____

निवासी _____

इस शोधकार्य **"MYELIN WATER AND PERFUSION IMAGING IN NORMAL APPEARING WHITE MATTER IN PATIENTS WITH MULTIPLE SCLEROSIS"** में भाग लेने के लिए सहमत हूँ। मैं इस शोधकार्य में भाग लेने के लिए अपनी स्वेच्छा से तैयार हूँ। मैं पुष्टि करता/करती हूँ की यह स्वीकृति देने के लिए मुझे कोई आर्थिक प्रलोभन नहीं दिया गया और न ही दबाव डाला गया है। मुझे इस शोधकार्य का उद्देश्य स्पष्ट कर दिया गया है और मुझे यह विश्वास है की यह विज्ञान और मानवता के लाभ के लिए है। मुझे इस शोधकार्य में होने वाले जोखिम के बारे में समझा दिया गया है और मेरी समझ में यह जोखिम बहुत मामुली है।

मैं इस शोधकार्य में भाग लेने के सम्बन्ध में किसी भी समय अपनी अस्वीकृति व्यक्त कर सकता/सकती हूँ और मेरे अस्वीकृति व्यक्त करने का मेरे इलाज पर कोई प्रभाव नहीं पड़ेगा।

_____ _____

गवाह के हस्ताक्षर रोगी के हस्ताक्षर/अंगूठे का निशान

नाम: नाम:

_____

अन्वेषक के हस्ताक्षर

_____

विद्यार्थी के हस्ताक्षर

ਮਰੀਜ਼ ਰਜ਼ਾਮੰਦੀ ਫਾਰਮ

ਮਿਤੀ_____

ਮੈ_____ਪਤਨੀ/ਪੁੱਤਰ/ਪੁੱਤਰੀ_____

ਵਾਸੀ _____

"MYELIN WATER AND PERFUSION IMAGING IN NORMAL APPEARING WHITE MATTER IN PATIENTS WITH MULTIPLE SCLEROSIS" ਤੇ ਚਲ ਰਹੀ ਖੋਜ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਰਜ਼ਾਮੰਦ ਹਾਂ। ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਮੈ ਆਪਣੀ ਮਰਜ਼ੀ ਨਾਲ ਤਿਆਰ ਹਾਂ। ਮੈ ਇਹ ਦਸਣਾ ਚਾਹੁੰਦਾ ਹਾਂ/ ਚਾਹੁੰਦੀ ਹਾਂ ਕੇ ਇਹ ਰਜ਼ਾਮੰਦੀ ਦੇਣ ਵਾਸਤੇ ਮੈਨੂ ਕਿਸੇ ਵੀ ਤਰਾਂ ਦਾ ਲੋਭ-ਲਾਲਚ ਨਹੀ ਦਿੱਤਾ ਗਿਆ ਅਤੇ ਨਾ ਹੀ ਕੋਈ ਦਬਾ ਪਾਇਆ ਗਿਆ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਦਾ ਮਨੋਰਥ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਭਰੋਸਾ ਦਵਾਇਆ ਗਿਆ ਹੈ ਕੇ ਇਹ ਖੋਜ ਵਿਗਿਆਨ ਅਤੇ ਮਾਨਵਤਾ ਦੀ ਭਲਾਈ ਲਈ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਵਿਚ ਹੋਣ ਵਾਲੇ ਖਤਰੇ ਬਾਰੇ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਯਕੀਨ ਹੈ ਕੇ ਇਹ ਖਤਰਾ ਬਹੁਤ ਮਾਮੂਲੀ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਦਾ ਢੰਗ – ਤਰੀਕਾ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ।

ਮੈ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਬਾਰੇ ਆਪਣੀ ਰਜ਼ਾਮੰਦੀ ਕਿਸੇ ਵੀ ਸਮੇ ਵਾਪਸ ਲੈ ਸਕਦਾ ਹਾਂ/ ਸਕਦੀ ਹਾਂ ਅਤੇ ਮੇਰੀ ਰਜ਼ਾਮੰਦੀ ਵਾਪਸ ਲੈਣ ਦਾ ਮੇਰੇ ਇਲਾਜ ਤੇ ਕੋਈ ਅਸਰ ਨਹੀ ਹੋਵੇਗਾ।

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ਗਵਾਹ ਦੇ ਹਸਤਾਖਰ ਮਰੀਜ਼ ਦੇ ਹਸਤਾਖਰ

ਮਿਤੀ_____ ਮਿਤੀ_____

ਪਤਾ _____ ਪਤਾ _____

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ਨਿਰੀਖਕ ਦੇ ਹਸਤਾਖਰ

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ਸਿਖਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ